LIGHT AND LIFE III

by

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Copenhagen has been a hospitable mecca for scientists for many decades. Foremost among the institutions attracting the aspiring young scientists from all the world have been the Carlsberg Laboratory and the Institute for Theoretical Physics. I have been fortunate in being close to both, though at different times. The transition from physics to biology was catalyzed by a lecture of NIELS BOHR's at 10:00 a.m. on August 15, 1932. It was the opening lecture of an International Congress of Light Therapists taking place rather solemnly in the Rigsdag, in the presence of your then Crown Prince and your then Prime Minister, STAUNING, and of many distinguished looking gentlemen from all parts of the world. I had arrived that morning by the night train from Berlin, as I have this morning, and I had been met at the station by LEON ROSENFELD who took me to breakfast and told me that BOHR wanted me to hear his lecture. Even though the immediate effect of the lecture was somewhat hilarious due to a comic mishap worthy of CHARLIE CAPLIN, the ultimate effect was to change the course of my life.

In this lecture, entitled »Light and Life,« BOHR proposed the bold idea that life might not be reducible to atomic physics. He suggested that there might be a complementarity relation between life and atomic physics analogous to the complementarity encountered with the wave and particle aspects of atomic physics. The result would be a kind of uncertainty principle regarding life, analogous to Heisenberg's uncertainty principle in quantum mechanics. The crucial passage reads as follows (2):

> »Thus, we should doubtless kill an animal if we tried to carry the investigation of its organs so far that we could describe the role played by single atoms in vital functions. In every experiment on living organisms, there must remain an uncertainty as regards the physical conditions to which they are subjected, and

> the idea suggests itself that the minimal freedom we must allow the organism in this respect is just large enough to permit it, so to say, to hide its ultimate secrets from us.«

This conjecture did not sit well with the biologists and especially the biochemists, but I became intrigued with it and motivated to learn biology and eventually to become a biologist. Thirty years later in 1962 when I had the privilege of organizing a Genetics Institute in Cologne, I invited BOHR to give the dedicatory lecture and suggested that he use this opportunity to reassess the point of view first broached in 1932. He accepted this challenge with enthusiasm and in this lecture, entitled »Light and Life Revisited,« he discussed the reducibility question in the light of the explosive progress in biochemistry and especially in molecular genetics that had taken place in the intervening decades.

He rephrased the original conjecture in the following way (3):

> » . . . it appeared for a long time that the regulatory functions in living organisms, disclosed especially by studies of cell physiology and embryology, exhibited a fineness so unfamiliar to ordinary physical and chemical experience as to point to the existence of fundamental biological laws without counterpart in the properties of inanimate matter studied under simple reproducible experimental conditions. Stressing the difficulties of keeping the organisms alive under conditions which aim at a full atomic account I therefore suggested that the very existence of life might be taken as a basic fact in biology in the same sense as the quantum of action has to be regarded in atomic physics as a fundamental element irreducible to classical physical concepts.«

The mysteries of life, in those days, from the point of view of physics, were indeed stark: cell physiologists had discovered innumerable ways in which cells responded »intelligently« to influences from the environment, and embryologists had demonstrated such feats as each half of an embryo developing into a complete animal! Such findings were vaguely reminiscent of the »wholeness« of the atom, of the stability of the stationary states. The stability of the gene and the algebra of genetics suggested something akin to quantum mechanics. The resistance of biologists to such ideas did not surprise BOHR. He had met the same resistance to the complementarity view of atomic physics among his physics colleagues. It is well known that both EINSTEIN and SCHRÖDINGER could never be shaken from their conviction that a return to the mechanistic concepts of classical physics would eventually occur.

Indeed, we might say that the discovery of the Double Helix in 1952 (39) did for biology what many physicists had longed for in atomic physics: a resolution of all the miracles in terms of classical mechanical models, not requiring an abdication of our customary intuitive expectations. The Double Helix, indeed! With one blow the mystery of gene replication was revealed as a ludicrously simple trick, making those who had expected a deep solution feel as silly as one might feel when shown the embarrassingly simple solution to a chess problem one may have struggled with in vain for a long time. The Double Helix, indeed! It does not matter that the mechanics of replication of nucleic acid has turned out to be enormously more complex than was thought in the first flush of victory and that even now vast uncertainties remain in this most basic area of molecular biology. Never mind! We now understand that organisms can be viewed very successfully as molecular systems, of enormous complexity, to be sure; nevertheless, an upper limit can be set to the complexity and ever more powerful methods to probe it are being developed at a mind-boggling rate.

Unfortunately, only an early version of BOHR's lecture of 1962 could be published. BOHR died on November 18, 1962, before completing the final editing. The final editing of a lecture, in BOHR's case was a process that continued for months, sometimes years, after a lecture. Some of us struggled with BOHR, dispassionately or not so dispassionately, about these editorial agonies: about how long a sentence should be in German, and especially in English. It was always in vain. It is my belief that these agonies

were counterproductive in the extreme. Countless potential readers were deterred by the style; only a handful struggled with BOHR's lectures to get out of them what he put in.

The present lecture will not relate at all to the earlier epistemological question. This question is by now a dead issue in the area of ordinary biochemistry and physiology, and it has not yet become a live issue in the area of psychobiology. I will, instead, address myself literally to Light and Life, to wit, to the question of how and when the various basic photochemical reactions involved in life have come about and what they do.

Let me first introduce the cast of characters.

Chlorophyll
Protochlorophyll
Retinal
Phytochrome
Cryptochrome (blue light receptor)
Photoreactivating Enzyme

Chlorophyll is shown together with heme in Fig. 1, championed by their respective partisans. Which should take evolutionary precedence? The flat rigid tetrapyrrole molecule chelates in the case of heme with iron; it is the function of the iron of this molecule to accept an electron from a donor and to transmit it to an acceptor. Heme does not function as a photoreceptor molecule in living organisms. Its best known role is that of electron transport in the respiratory chain of oxidative phosphorylation performed in bacteria and in mitochondria.

The same flat rigid tetrapyrrole is chelated in chlorophyll with magnesium and here plays the central role in photosynthesis as carried out in green plants and in photosynthetic bacteria. This molecule, too, is in the business of electron transfer. The electron in question, however, does not belong to the chelated metal but to the delocalized system of ring electrons and indeed not to one ring but to a special pair of chlorophylls (28,34). Upon arrival of a quantum

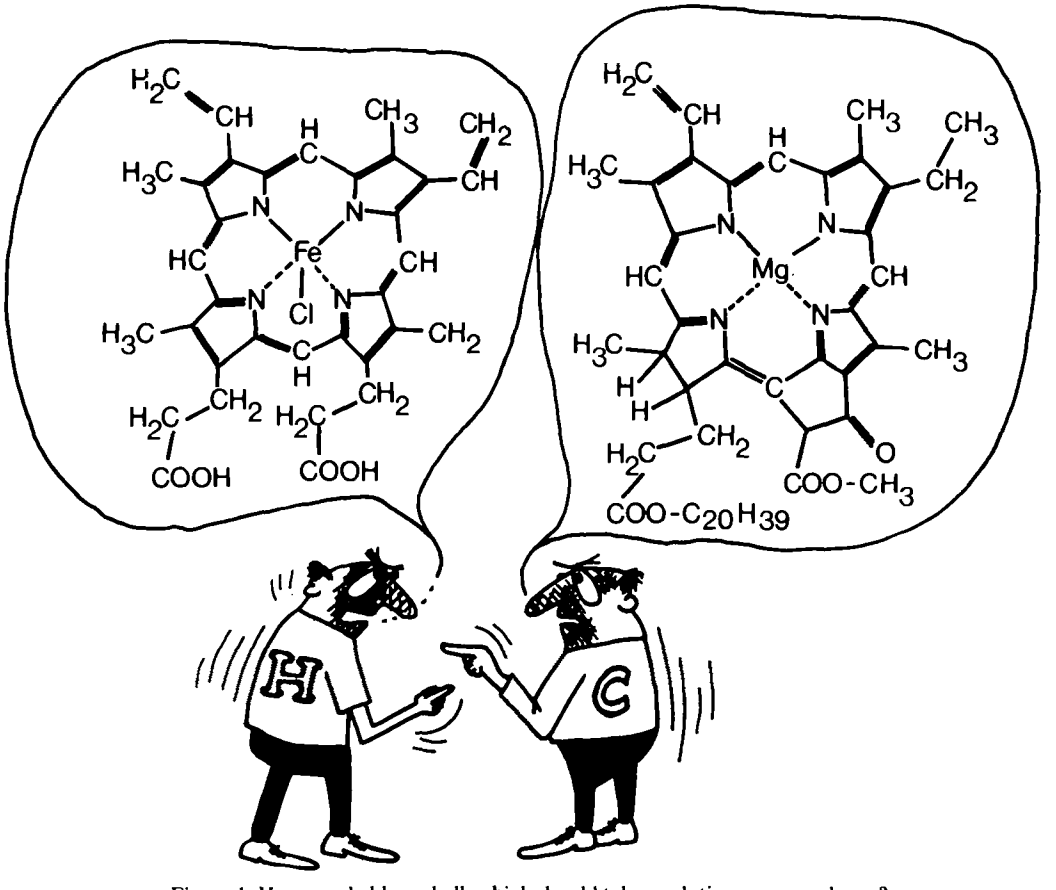

Figure 1. Heme and chlorophyll: which should take evolutionary precedence?

of light in a photosynthetic reaction center, an electron is removed from the chlorophyll special pair (the so-called primary electron donor) and quickly ($\sim 10^{-11}$ seconds) transferred to what is thought to be, at least in the case of photosynthetic bacteria, a nearby chlorophyll lacking magnesium (33,8). From there it is handed within a fraction of a nanosecond still further to the so-called primary acceptor, now believed in the case of photosynthetic bacteria to be a complex of a nonheme iron and a quinone (31). The complex structure of the reaction center is designed to perform this elegant maneuver of charge separation and to prevent back reaction. The rigid frame of the porphyrin is ideally suited to permit this maneuver. To complete the duty cycle of the reaction center the loss of the electron from the chlorophyll special pair to the acceptor is followed by replacement of an electron from a donor, a *c*-type cytochrome in the case of photosynthetic bacteria.

Because the nature of the photosynthetic reaction center is less complex in bacteria than in green plants more details are known about the primary photochemistry in the former. Green plant photosynthesis involves the coupling of two photosystems, each with its own primary electron donor and acceptor. Here, too, the evidence for a chlorophyll special pair as the primary electron donor is fairly good for photosystem I but less clear for photosystem II. The primary acceptor in photosystem I is thought to be a ferredoxin (iron-sulfur protein). The nature of the photosystem II primary acceptor is still obscure (1).

The net effect, then, with chlorophyll in photosynthesis and with heme in respiration, is electron transport, in photosynthesis driven by light, in respiration spontaneous. Which is older? That the two molecules are closely related in their evolution is attested not only by their structure but also by their biosynthetic pathways. These pathways are diagrammed in a very simplified way in Fig. 2.

It is a new finding (10, 11) that the key interme-

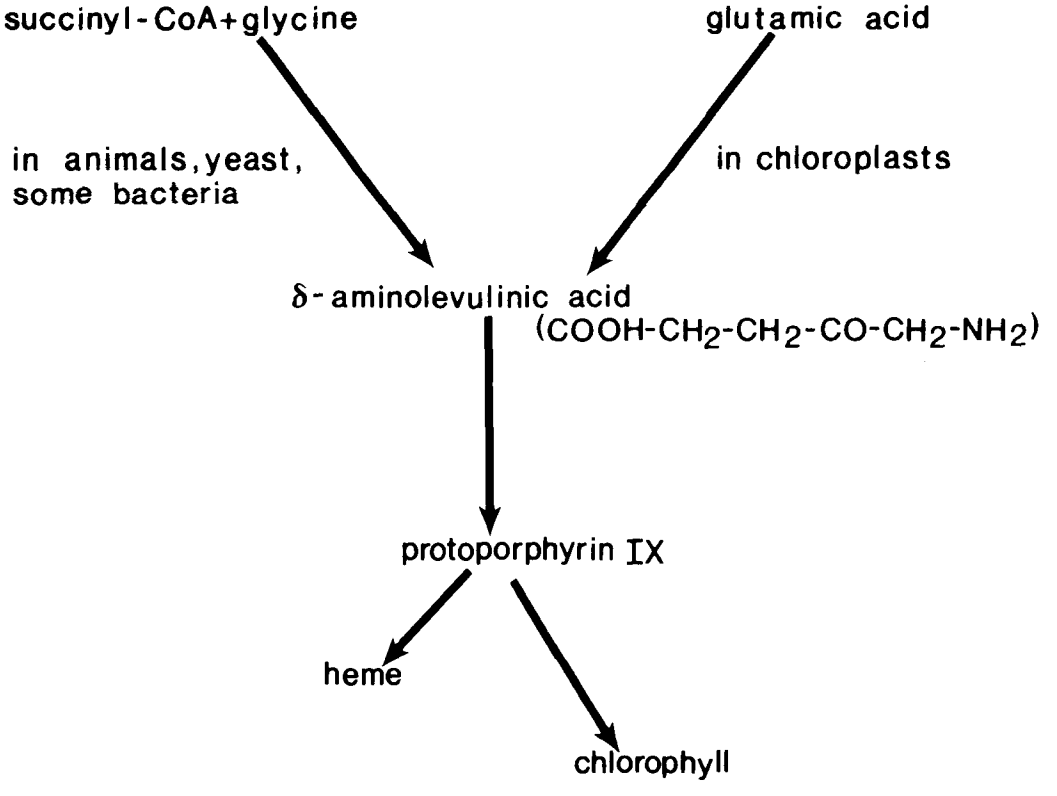

Figure 2. In the biosynthesis of heme and chlorophyll the precursor δ-aminolevulinic acid is synthesized by different pathways in chloroplasts and in cytoplasm.

diate δ-aminolevulinic acid is synthesized by different pathways in chloroplasts and in cytoplasm. In chloroplasts it serves as precursor of both chlorophyll and heme, in cytoplasm of heme. The chloroplast pathway presumably dates back to the cyanobacteria (blue-green algae), the cytoplasmic one to some other prokaryotic ancestor.

Which is older? We might try to answer this question by looking at the functions the two molecules serve. Not long ago we would have voted unquestionably for chlorophyll, arguing that oxidative phosphorylation and its electron transport could only have come into the living world after oxygen got into the atmosphere, and oxygen got into the atmosphere beginning with the appearance of photosynthesis in the cyanobacteria. The argument, however, is not so clear-cut as all that since heme and heme electron transport are not limited to oxidative phosphorylation. Especially in bacteria it occurs in a bewildering variety of contexts quite unrelated to oxygen and instead closely related to photosynthesis itself. More interesting still is the class of c_3 cytochromes containing 4 hemes per molecule and very little protein indeed: hardly enough protein to wrap up the 4 hemes separately (25,6)! These strange cytochromes, found so far only in bacteria which use sulfate instead of O_2 as electron acceptors, can store 4 electrons. Their function is unknown but they look more like storage devices than transport devices.

In connection with chlorophyll we must mention a closely related molecule involved in a physiologically important photochemical reaction. In the final step of chlorophyll synthesis two hydrogen atoms are added to ring IV of *protochlorophyll.* The prolamellar body membranes in the plastids of dark grown seedlings contain protochlorophyll in a protein complex such that protochlorophyll is reduced to chlorophyll (with hydrogen from an unidentified donor) only upon absorption of light by the protochlorophyll. The conversion occurs with a high quantum yield and in higher plants is absolutely dependent on light. This photochemical reaction controls the conversion of the prolamellar bodies to thylakoids. The events following the excitation of protochlorophyll are very incompletely understood (15, 27, 16).

Thus we must leave the two gentlemen to their argument and move on to the next character, *retinal* (Fig. 3). This well-known prosthetic

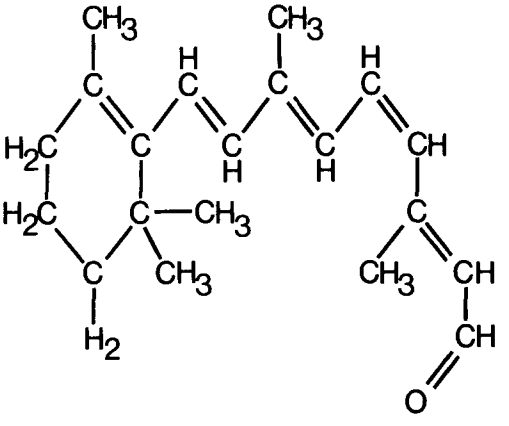

Figure 3. 11-cis-retinal: the chromophore of the animal visual pigment rhodopsin. The retinal is joined to the protein (opsin) via a protonated Schiff base linkage to the C_{15} of retinal. In animals, the action of light on 11-cis-retinal is to cause an isomerization to the all-trans form followed (in vertebrates) by dissociation of the retinal and opsin. In invertebrates no such dissociation takes place. The reversal to 11-cis-retinal is also a photoreaction. The retinal in bacteriorhodopsin is 13-cis rather than 11-cis and the action of light does not necessarily result in a cis-trans isomerization.

group common to the visual pigments of all animals performs its function not by gaining or losing an electron but a light-induced cis to trans isomerization (38). This then forces a conformational change on its protein carrier (opsin) and thereby causes some alteration in the properties of the membrane in which the protein is embedded. Until very recently it was believed that retinal-opsin photoreceptors occurred exclusively in animals and that the primary photochemical reaction was cis-trans isomerization of the retinal. However, a few years ago STOECKENIUS and OESTERHELT (29) made the startling discovery of a retinal protein photoreceptor in a class of bacteria (the *Halobacteria)* and LILY JAN (20) showed that the duty cycle of this so-called bacteriorhodopsin does not necessarily involve an isomerization of the retinal! More startling still, it was dis-

covered that the absorption of light by bacteriorhodopsin results in a proton translocation from one side of the purple membrane to the other, thus creating a pH gradient which then drives the synthesis of ATP (30). Thus, with a single blow, retinal photochemistry has been elevated from the specialized and evolutionarily late function of animal vision to the most basic energy metabolism: a primitive photosynthesis in bacteria. Rightfully, therefore, we might add Mr. OESTERHELT'S and Mr. STOECKENIUS' portraits to that of the two gentlemen espousing heme and chlorophyll, respectively.

In addition to its role in energy metabolism, bacteriorhodopsin acts as the photoreceptor for a light controlled tactic response of *Halobacteria.* A sudden decrease in light intensity will evoke a reversal in the swimming direction of this organism. The action spectrum of this response shows a maximum at 565 nm, corresponding to the peak of absorbance of bacteriorhodopsin in the visible (17). Thus bacteriorhodopsin appears as the only photoreceptor molecule known to play roles both in energy generation and behavioral responses.

Our fourth character, *phytochrome* (Fig. 4), (35) we can afford to treat a little more lightly and I would like to treat it in conjunction with the other plant visual pigment, *cryptochrome.* Here is a comparison of the two pigments. Cryptochrome is a blue light receptor; phytochrome is sensitive to red and far red light. Cryptochrome is found widely in plants, fungi and bacteria; phytochrome appears to be limited to higher plants only. Cryptochrome is responsible for phototropism, for phototaxis, for many cases of photodifferentiation and for the control of respiration; phytochrome is involved in a plethora of morphogenetic controls of higher plants.

The chemical identity of cryptochrome was moot until very recently. For at least 20 years β-carotene and flavin have been contenders (Fig. 5). However, for the fungus *Phycomyces* we have been able to provide strong evidence that the physiological photoreceptor is not β-carotene (32) and, in fact, is a flavin (5). The evidence against β-carotene comes from the photophysiology of mutants blocked in two of the biosynthetic steps leading to β-carotene. These mutants contain essentially no β-carotene (less than 10^{-5} of the amount of β-carotene found in the wild type) and yet these mutants exhibit normal tropic responses to light down to the very lowest intensity that wild type can detect.

The evidence in favor of flavin derives from the fact that flavin has its lowest triplet state at

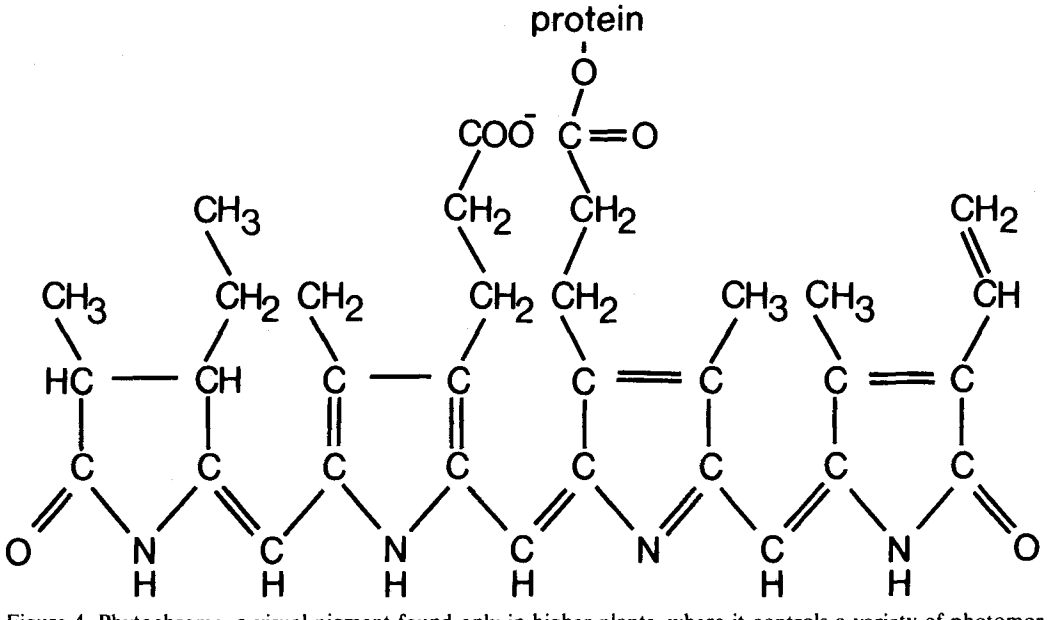

Figure 4. Phytochrome: a visual pigment found only in higher plants, where it controls a variety of photomorphogenetic responses. It is believed to function via a light-induced conformational change.

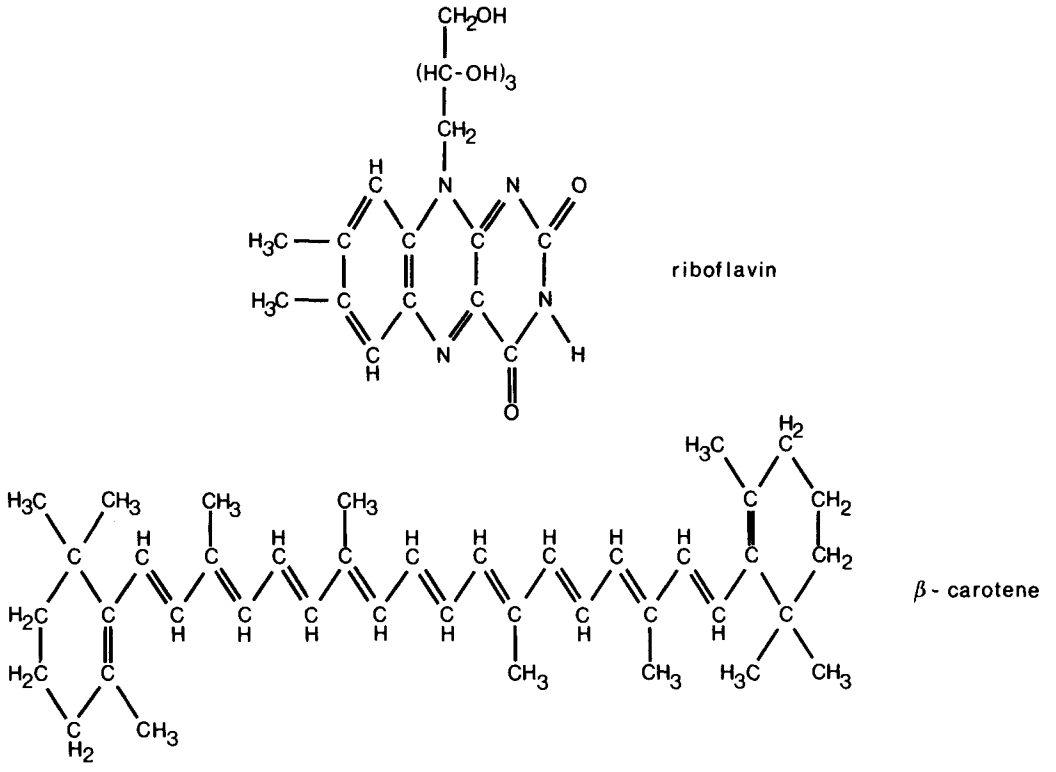

Figure 5. Riboflavin and β-carotene: two long-time candidates for cryptochrome, a photoreceptor occurring in a variety of fungi, plants and bacteria.

about 600 nm (36) (the corresponding triplet state of β-carotene is in the infrared (24)). It turns out that normal physiological photoresponses in sporangiophores of *Phycomyces* can be elicited by illumination with orange light. The action spectrum for photoresponse to orange light has a maximum at about 595 nm. The intensity needed is 10^8 times higher than that needed for the response elicited by blue light. This factor, 10^8, corresponds to the degree of forbiddenness of the direct optical transition from the ground state singlet to the lowest triplet state. This indication of flavin as the receptor pigment hopefully will soon be followed by more incisive insights into its mode of functioning. Since the flavins are typically in the redox business, a reasonable guess may be a charge separation, as in photosynthesis, and a very special structure may be needed, here too, to prevent back reaction. Or it might be a 2 electron (hydride) reduction. How either of these events then serves as a signal, throwing a switch in the cell's activities, is today still totally obscure.

The structure of phytochrome has been reasonably certain for a number of years: an open chain tetrapyrrole covalently linked to a protein. The mechanism here appears to be a flip-flop between two isomeric states, driven in one direction by red light (665 nm), in the other direction by far-red light (730 nm). The flip-flop proceeds through a series of intermediate states, different ones in the two directions (flip versus flop).

For cryptochrome we known that it measures light *intensity:* sometimes relative light intensity from two directions as in phototropism, sometimes absolute light intensity as in photocarotenogenesis. If it measures relative light intensity it can do so down to exceedingly low light levels, involving a mechanism of adaptation to various light levels over the incredible range of 9 decades! The function of phytochrome appears to be to measure light *quality,* especially sunlight versus shade, by assessing the relative intensities of red and far red light (26).

As long as phytochrome is found only in

higher plants I do not think that any gentleman can be found desiring to argue in favor of its evolutionary priority. for cryptochrome the situation is different. Flavin is one of the most ancient molecules and blue light responses are widespread in prokaryotes. However, since the nature of cryptochrome is only now becoming clear, no biochemist has yet risen to its defense. Our final character, the *photoreactivating enzyme* (12), occupies a special position by its extraordinary elusiveness. Photoreactivation was discovered in 1949, simultaneously by KELNER in bacteria (21, 22) and by DULBECCO in phage (7). It consists of light-induced repair of DNA damaged by ultraviolet light.

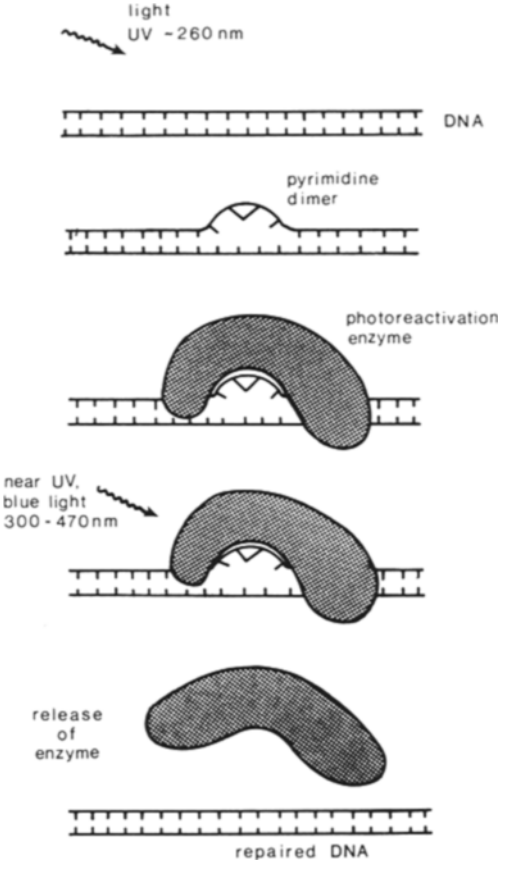

Figure 6. Scheme for photoreactive repair of DNA damaged by ultraviolet light. Far UV light causes the formation of dimers between adjacent pyrimidine nucleotides. Near UV and blue light supply energy for the enzymatic repair of this structural damage.

Photoreactivation constitutes only one of several mechanisms of repairing damaged DNA. Photoreactivation specifically unhitches neighboring pyrimidines stuck together (dimerized) by the action of far ultraviolet light (Fig. 6). Thus, this enzyme is the only photopigment for which we can exactly define its effect to the end. The mechanism of this unique photoenzymatic catalysis has if anything become more mysterious in recent years. That we are dealing with an enzyme has been clear for many years. But the enzyme is present in very few copies in the cell and is difficult to handle. It combines reversibly with its substrate, the pyrimidine dimer in damaged DNA. The dimer is a cyclobutane complex between the two bases. Upon absorption of light in the blue and near ultraviolet this enzyme-substrate complex comes apart into enzyme and repaired DNA. Recently the gene for this protein has been manipulated into the phage lambda and thus it has been possible to enormously increase the production per cell. Purified preparations have been obtained which indicate a molecular weight of about 60,000 daltons. It is found that the purified enzyme does not absorb at the wavelength at which it acts when it combines with DNA! An appropriate absorption band does appear upon mixing enzyme with UV irradiated DNA. (37,40). What kind of a complex of an enzyme with DNA could produce an absorption band of this color? I do not think anybody would here venture a conjecture at the present time. (Quite possibly the reaction scheme is a little more complicated than indicated in Fig. 6. There is evidence for two forms of the enzyme in yeast, PRE_1 and PRE_2; PRE_1 is the one usually studied. But it can be converted to PRE_2 by near ultraviolet or 570 nm light! PRE_2 has three times higher activity. PRE_2 reverts *very* slowly (many hours) to PRE_1 (14,13)).

Photoreactivation is a phenomenon common to both prokaryotes and eukaryotes. It is found in every kind of organism. Quite possibly this enzyme represents the oldest of all photochemical reactions invented by life. The intensity of ultraviolet light at the surface of the earth was very higher before oxygen got into the atmosphere and the ozone layer was formed. Du-

ring these early eras repair of UV damage of DNA must have been of paramount importance. We may therefore confidently expect that a gentleman or lady rooting for this enzyme will appear as soon as its chemistry opens up.

This short list of six molecules--chlorophyll, protochlorophyll, retinal, cryptochrome, phytochrome, and photoreactivating enzyme--exhausts the list of compounds whose photochemistry is relevant for biology, and has been relevant for a very long time.

Why is this list so short? Why has Life settled on so few molecules to do its photochemistry when it seems to have used an almost limitless variety for the biochemistry without light? To begin such a discussion we must first remark that a quantum of visible light, say 500 nm, represents a very large chemical energy, about 2 eV, or 46,000 cal/mole. To throw such quantities of energy around in any random way must not be permitted. Specifically, many excited molecules can deliver their fateful energy to oxygen and easily do so in two quite distinct ways. Such a molecule may donate an electron to molecular oxygen and create the very reactive superoxide anion, O_2^-. To dispose very rapidly of this dangerous free radical, Life invented at a very early stage enzymes, called superoxide dismutases (9), which catalyze the conversion of $2\ O_2^- + 2H^+$ to $O_2 + H_2O_2$. *Or,* the excited molecule can deliver its energy to oxygen by raising the oxygen to the metastable singlet state, which in turn can cause havoc. Indeed, the role of carotenes in some organisms is to pick up and degrade this energy from the singlet oxygen (23). Quite generally, therefore, we must expect that pigment molecules in aerobic cells must be constructed so as to minimize the deleterious effects of a large quantum of light energy.

Still, the question remains, why is our list so short? What is so special about these five molecules that Life has found them to be peculiarly useful? We might even be tempted to shorten the list by lumping phytochrome with chlorophyll, since both are tetrapyrroles. From the point of view of molecular evolution this lumping would be justified: biosynthetically these two substances are first cousins. But from the point of view of photochemical mechanics they are worlds apart: chlorophyll in the photosynthetic reaction center serves charge separation. An electron is moved from one side of the thylakoid membrane to the other, a large distance (~40 Å). HOPFIELD (19) has shown that the rigidity of the flat tetrapyrrole ring and its large π-orbital system are essential for this function. By rigidity we mean that the framework of atomic positions does not shift much when an electron is removed. This rigidity is essential for fast transfer of an electron from the special-pair chlorophyll to pheophytin, the first step of the light-induced charge separation in bacterial photosyntheses. This transfer can best be thought of in terms of quantum mechanical tunnelling (18). The large π-orbital system permits the separation over 10 Å in one blow. In contrast, phytochrome does not serve charge separation at all. Instead, it imposes a conformational change on a membrane protein, thereby (presumably) permitting the translocation of other control molecules through the membrane. The contrast could not be greater: instead of the rigidity of chlorophyll the flexibility of phytochrome is essential, and the large systems of π-orbitals, instead of facilitating long distance electron transport, merely serve to create an adjustable absorption band at relatively low quantum energy in the red or far red.

In both these respects phytochrome is strikingly similar to retinal: here, too, the flexibility of the molecule serves to enforce a conformational change in the carrier protein (again probably controlling a membrane permeability) and again the absorption band is tunable by interaction with various opsin proteins, thus creating visual pigments for many colors.

It should be noted, further, that retinal was not tailor-made by Life for the purpose of photochemistry. Chemically it is vitamin A aldehyde, and a half-β-carotene, both multipurpose molecules of general biochemistry. And yet, Life chose it for all of animal vision and for the seemingly unrelated function of proton translocation in bacteria. At this point it is not clear whether or not the bacterial and the animal rhodopsins are evolutionarily related. In both cases retinal is attached to the ε-amino

group of a lysine. But the peptides containing this lysine show no similarity (4).

For cryptochrome, as a photopigment, the most striking thing is its unoriginality as a molecule. The flavin structure, as FMN and FAD, is by far the most widely used of all the molecules of biochemistry. Every cell contains dozens of different enzymes with flavin in the prosthetic group. This great variety is however a little deceptive. The types of reactions performed by these flavoenzymes are few (one- *and* two-electron transfers and O_2 activation); the variety lies in the manifold of substrates. We should be prepared to find a similar variety with respect to cryptochrome, i.e., there might be many photoflavoenzymes each with a different specificity for doing the same thing to a different substrate.

Thus we are left with the impression that Life, in its choice of molecules to do photochemistry with, has been extremely conservative and unoriginal. It has taken odd molecules lying around and used them with utmost skill to construct devices of high specificity, reliability, efficiency and sensitivity. Under these circumstances it may turn out that scientists now, knowing so much more about photochemistry than Nature ever dreamed of, can construct devices, based on new molecules, that outdo the feats of organisms. In some respects that is true already for some current solid state devices. This discussion of Light and Life has been strictly limited to photo*chemistry*. I have excluded the auxiliary pigments employed as antenna molecules in photosynthesis, to absorb light and to transfer it to the reaction centers. These molecules do not undergo photochemical changes themselves; they sensitize photochemical changes in one of those listed. Similarly, I have excluded the vast number of coloring and screening pigments which modify reflected or transmitted light by absorption, without concomitant chemistry, as well as those sophisticated structures which create colors by physical means: interference, refraction or diffraction. All these topics rightfully also belong to the topic »Light and Life«; they serve important functions and are very much subject to evolution. In this lecture I merely wanted to draw attention to the very few molecules whose photochemical reactions play essential physiological roles. I am afraid the few comments I was able to offer have done no more than to accentuate the »puzzle of the short list.«

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