Review

Melatonin in the evolution of plants and other phototrophs

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Running title: Melatonin and plant evolution

Received: May 17, 2019; Accepted: June 20, 2019

ABSTRACT

Melatonin is present in numerous phototrophic eukaryotes, not only in plants in the meaning of Archaeplastida or of Viridiplantae. It is also formed in members of other superclades, such as Excavata and SAR clade. Typically, their respective phototrophs have acquired chloroplasts from phototrophic eukaryotes, either by taking them up as endosymbionts or by chloroplast capturing. It has been the aim of this overview to trace the phylogenetic relationships between the various phototrophs according to actual, genetically based taxonomy. This includes the consideration of primary heterotrophs that also exist within the same groups and some secondary heterotrophs that have lost functional chloroplasts. The presence of melatonin in these different taxa is discussed under the aspects of its cyanobacterial or α-proteobacterial origins, as transmitted by plastidial or mitochondrial ancestors, or by horizontal gene transfer. Peculiarities of melatonin metabolism that have evolved in some of these groups are also addressed.

Keywords: algae, bacteria, chloroplasts, circadian, melatonin, meta-algae, mitochondria, plants.

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1. INTRODUCTION

Melatonin is a phylogenetically old molecule (1-4). To date, its presence has been shown in various bacteria and almost all eukaryotes that have been tested for its existence. The evidence concerning archaeans is still insufficient, because the molecule itself has not been demonstrated and only indirect hints exist on the basis of sequence homologies of *N*-acetyl transferases (5, 6) which provide, however, no satisfactory information, for reasons to be discussed in the subsequent section.

The ubiquity of melatonin implies that it has to have properties of value to these manifold organisms, which differ so much in their morphology, physiology and biochemical peculiarities. This conclusion has two different aspects. First, there must exist a primary molecular property of general relevance. Second, on this basis, other more or less clade-specific functions should have developed in the course of evolutionary diversification. The primary function may be seen in its direct antioxidant actions by scavenging free radicals and other reactive intermediates (7). This property was impressively demonstrated by its superior capacity of detoxifying hydroxyl radicals

(8, 9). Later, various other endogenously formed, reactive toxins have been shown to be eliminated by melatonin, too (1, 10, 11).

The direct antioxidant effects by scavenging of free radicals and other oxidants such as singlet oxygen show, when analyzed for the chemical details and products formed thereby, remarkable peculiarities that make a substantial difference to other antioxidants. First, melatonin does not undergo redox cycling at substantial rates, contrary to compounds like ascorbate and other direct antioxidants (12). Second, the products formed from melatonin are, to the best of our knowledge, nontoxic and terminate radical reaction chains (13, 14). Of course, any interaction of a radical scavenger with a free radical generates a new free radical from the scavenger. However, in the case of melatonin, the melatonyl radicals rapidly undergo reactions with a second radical which terminates the reaction chain. This conclusion does not imply that a stable product formed from melatonin represents the end of catabolic pathway. On the contrary, the primary products, which can be either monohydroxylated derivatives (9, 15), in particular, cyclic 3-hydroxymelatonin (16, 17), or the product formed by pyrrole ring cleavage, N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) (13-15, 18), contribute themselves to the detoxification of free radicals. Efficient free radical scavenging by cyclic 3-hydroxymelatonin has been documented (19). Although AFMK is chemically more inert than other kynuric compounds (18), it does interact with hydroxyl radicals and, at lower rates, with other electron/hydrogen-abstracting radicals to also convey protective effects (20-22). As a result, a single molecule of melatonin can, by virtue of its products, detoxify several free radicals in a reaction cascade (21). This may end up in the elimination of 10 free radicals (22).

Notably, melatonin differs from other indolic compounds under the aforementioned aspects. This concerns especially the halting of radical reaction chains, despite the existence of a scavenging cascade, in which, again and again, indolic or kynuric radicals are turned into principally stable intermediates, which, nevertheless, can undergo new radical reactions. The difference is particularly evident when the oxidation chemistry of melatonin is compared with that of its precursor serotonin, but also obvious with other indolic metabolites (8, 14). In these cases, radical scavenging is followed by prooxidant reactions. Moreover, even those compounds that also have a high capacity of scavenging free radicals turn out to be detrimental if they promote radical reaction chains or generate toxic products. However, melatonin and the studied products of the scavenging cascade are biologically well tolerable and typically protective (19-21).

Another important point for the understanding of melatonin's primary roles in evolution as well as in its value to light-exposed organisms concerns its property as a photooxidizable compound. It can be converted to AFMK by either singlet oxygen [$O_2(^1\Delta_g)$] (7, 23) or in photocatalyst-mediated reactions (7, 24, 25). Meanwhile, melatonin has been investigated in terms of singlet oxygen scavenging efficiency and it was found to be rather potent relatively to other biological low-molecular weight compounds including the frequently used histidine. However, it was topped by one of its metabolites, N^1-acetyl-5-methoxykynuramine (AMK), a secondary product of AFMK (26). The property of being oxidized under the influence of light gave rise to the assumption that melatonin may have been a particularly suitable candidate in the evolution to mediate the information of darkness, a condition under which it is more stable (24, 27).

Following the evolutionary routes of melatonin in phototrophs requires a look back to its prokaryotic origins. While melatonin, being a small but obviously highly valuable compound, has not been matter to evolutionary changes, differences in its biosynthetic pathway exist and relatively substantial variations between organisms have developed concerning the details of its catabolism (15). These aspects will be also addressed in the present article.

2. ON THE PROKARYOTIC ORIGIN AND HORIZONTAL GENE TRANSFER

Melatonin synthesis can be traced back to bacteria. This compound has been demonstrated in several bacterial taxa, such as α-proteobacteria [*Rhodospirillum rubrum* (28), *Erythrobacter longus* (29)], γ-proteobacteria [*Escherichia coli* (30)] and cyanobacteria [*Spirulina platensis* (31, 32)]. With regard to eukaryotes and specifically to plants, cyanobacteria and α-proteobacteria are of particular importance. Cyanobacteria are ancestors of plastids and, in fact, melatonin synthesis was demonstrated in chloroplasts (33). In fact, a key enzyme of melatonin formation, serotonin *N*-acetyltransferase (SNAT) from *Oryza sativa* has a substantial homology to that of the cyanobacterium *Synechocystis* and is, notably, plastidially located (34, 35). To date, all SNAT isoenzymes of plants *sensu stricto* have been reported to be located in chloroplasts (33). Although other enzymes of the melatonin synthetic pathway have been also found in various cellular compartments, a cyanobacterial origin of plant melatonin formation is highly likely. However, an additional contribution of a second bacterially derived source of melatonin should not be left out of sight. α-Proteobacteria are regarded as the ancestors of mitochondria and the formation of melatonin in this group (28, 29) indicates that eukaryotes have inherited melatonin from these organisms, too (37, 38). This view is consistent with the presence of AANAT (aralkylamine *N*-acetyltransferase) homologs in bacteria (39). Importantly, AANAT catalyzes the same reaction as SNAT, but differs from the latter in gene structure and sequence. In fact, melatonin has been also found in plant mitochondria (36). Moreover, the recent findings of melatonin formation in animal mitochondria (40) has directed considerable attention to the α-proteobacterial origin of melatonin. The α-proteobacterial heritage of melatonin formation cannot be directly deduced from the species in which this compound was first discovered, *Rhodospirillum rubrum* and *Erythrobacter longus*, which are not direct ancestors of mitochondria. These ancestors have presumably to be sought in the family of the Rickettsiaceae, since genome comparisons between the giant mitochondria of the jakobid flagellate *Reclinomonas americana* revealed highest homology with *Rickettsia provazekii* (41). Nevertheless, the presence of melatonin in the α-proteobacterial clade may indicate that rickettsiaceans including the mitochondrial ancestors are/have been capable of synthesizing melatonin.

A driving force for the use of melatonin by bacteria may be seen in the invention of photosystem II by cyanobacteria, which represented a switch from primitive forms of nonoxygenic photosynthesis and resulted in the oxygenic type. Thereby, the atmosphere of the earth was profoundly altered. The increase of oxygen concentration that also occurred in aqueous habitats was a life-threatening process to all exposed organisms. Of course, cyanobacteria also had to protect themselves against oxygen toxicity and respective mechanisms of oxygen management had to be present before they were able to produce oxygen at high rates. One might speculate whether protective molecules were already present prior to the development of photosystem II, perhaps, for coping with high UV intensities in upper water layers, at times when no ozone layer could shield organisms from this radiation. In this case, damage by UV light would have been different from that caused by reactive oxygen species. At least, in a transitory phase with still poor ozone shielding but sufficient oxygen levels to generate oxygen free radicals and singlet oxygen, protection against oxidative damage became increasingly important.

What remains unclear is the specific taxon in which melatonin was first “invented”. One might speculate whether cyanobacteria, which had developed oxygenic photosynthesis, had first the need of producing this antioxidant, but this is not certain, since oxygen management by a combination

of enzymatic mechanisms and other low molecular weight antioxidants might have been sufficient, although the production of melatonin presumably conveyed an additional selection advantage. However, the need of developing efficient antioxidative protection mechanisms was likewise present in all other, previously anaerobic organisms exposed to oxygen. The genes of the biosynthetic machineries for melatonin formation may have entered other bacteria and even cyanobacteria by horizontal gene transfer, which is a frequently observed phenomenon among these organisms. In reverse, respective genes may have been transferred from cyanobacteria to other taxa. However, when assuming horizontal gene transfer between the different bacterial groups, a problem remains concerning the serotonin and/or 5-methoxytryptamine *N*-acetylating enzymes. If we heed the sequence differences between SNAT and AANAT, these two forms of enzymes are not aliases, though one can sometimes read such a statement. If we, therefore, take for granted that the plastidial SNAT of Viridiplantae is derived from the cyanobacterial enzyme (34), and if we conclude that AANAT has originated in other bacteria (39), e.g., in α-proteobacterial ancestors of mitochondria, an interchange of the respective genes appears less likely, but additive actions would be allowed.

Nevertheless, the acquirement of genes of melatonin formation by horizontal gene transfer remains to be a relevant possibility in bacterial evolution. Additionally, this aspect may be extended to another major domain of organisms, the archaeans. To date, direct evidence for melatonin formation is still missing in this group. However, sequence homologies were reported between archaeal and eukaryotic *N*-acetyltransferases and the archaeal enzymes were placed in the *N*-acetyltransferase family (4-6, 38). On the other hand, sequence homologies are not at all reliable criteria for functionality and substrate specificity. Even point mutations can profoundly alter the substrate specificity, as shown, e.g., in another group of enzymes with relevance to melatonin formation, the aromatic amino acid decarboxylases (42). It should be also remembered that several invertebrate AANATs are not involved in melatonin synthesis, but rather in sclerotization of integuments or formation of other neurotransmitters (43, 44). Melatonin synthesis in several invertebrates may be also explained on the basis of other *N*-acetyltransferases, such as orthologs of NAT-1, which was even reported to contribute to melatonin formation in the mammalian skin (45). Similarities between archaeal and bacterial sequences have been generally interpreted as being indicative of horizontal gene transfer (6, 38). This possibility of gene exchange between bacteria and archaea is supported by findings in mixed biofilms. Recent multispecies biofilms are characterized by a remarkable extent of intra- and interspecific communication, by both quorum and community sensing, and by the exchange of extracellular membrane vesicles (46), a prokaryotic phenomenon reminiscent of eukaryotic communication via exosomes. Despite the likelihood and, in cases of other genes, demonstrated horizontal gene transfer between bacterial and archaeal species, the direction of transfer remains often elusive. It should be remembered that archaea do not only contain anaerobic but also aerobic and facultatively anaerobic species, in particular among the halophiles (47, 48). Therefore, a need for coping with oxygen, also under exposure to high light intensities, exists in this group of archaeans. Researchers interested in archaeal melatonin may select respective halophiles for their analyses, instead of working with anaerobic and extremely thermophilic species that are more difficult to handle. Apart from the possibility of horizontal gene transfer, the presence of both archaeal and bacterial genes in eukaryotes may be deduced from a merger between an archaean ancestor and an α-proteobacterial endosymbiont. Although this has been often disputed, the actual view that has emerged from the discovery of the so-called Asgard Archaea tends to favor this eukaryotic origin (49). The

incorporation of cyanobacteria as ancestors of chloroplasts has to be seen as a later event that leads to eukaryotic phototrophs.

3. MELATONIN IN EUKARYOTIC PHOTOTROPHS OUTSIDE THE ARCHAEPLASTIDA

The traditional term of 'plants' in the meaning of a 'kingdom" at same rank with 'animals" has completely lost its justification by modern genetic and cell biological taxonomy. The Archaeplastida represent a subgroup of the major eukaryotic taxon ("supergroup") of the Diaphoretickes. The Archaeplastida contain three taxa of phototrophs, Glaucophyta, Rhodophyceae and Viridiplantae. For a definition of plants, the criterion of photosynthesis is evidently inadequate, not only with regard to the domain of bacteria, but also within the domain of eukaryotes. Numerous eukaryotic clades contain photosynthetic species, because of either primary endosymbiosis with descendants of cyanobacteria or secondary endosymbiosis with eukaryotes carrying chloroplasts. The latter mode of endosymbiosis has given rise to the term of 'meta-algae', to indicate that these clades have not been originally photosynthetic. Secondary endosymbiosis reveals itself by more than two plastidial membranes, e.g., three in euglenoids and many dinoflagellates, and four in most diatoms and in brown algae (50, 51). Such processes have occasionally occurred several times. For instance, the dinoflagellate *Kryptoperidinium foliaceum* carries a second nucleus with surrounding cytoplasm that belongs to a diatom-derived endosymbiont (52). Tertiary and even serial endosymbiotic events have taken place in a number of cases (52, 53). The sources of secondary or tertiary plastids can be different and result from interactions with green algae, diatoms or, in the case of *Chromera velia* (Chromerida), with a rhodophycean (53, 54). As an alternative to the uptake of an entire endosymbiotic eukaryote, like in *Kryptoperidinium*, chloroplast capture from other eukaryotic phototrophs seems to have occurred, too (51-53). This appears to be the case in many other dinoflagellates and in *Euglena*. On the other hand, the development of plastids because of primary endosymbiosis with cyanobacteria has not only occurred in Viridiplantae, but also in an amoeba, *Paulinella chromatophora* (55), and in a diatom, *Rhopalodia gibba* (56). Thus, the situation in diatoms and dinoflagellates indicates a degree of complexity that does not allow premature generalizations.

The diversity of plastidial origins in eukaryotic phototrophs outside the Viridiplantae raises the question of whether the association with secondary plastids may have implied the acquisition of genes of melatonin synthesis. As the eukaryotic hosts usually transfer many genes of their endosymbionts into the nucleus, this may appear more likely in cases in which an entire endosymbiotic eukaryote has been taken up rather than in cases of chloroplast capture. However, the capacity of producing melatonin can also be inherited from mitochondria in all eukaryotes that harbor these organelles. Taking the example of *Euglena*, which has been concluded to have acquired its plastids by capture and which is a member of the unranked eukaryotic major taxon ('supergroup') of the Excavata, melatonin formation has been unequivocally demonstrated (1, 30, 57, 58). *Euglena gracilis*, member of the subgroup Euglenozoa, was also reported to exhibit a circadian rhythm in melatonin concentration (57), but the first author who had collected these data never published the details. Although the intracellular source of melatonin in *Euglena* is to date unknown, its synthesis may not be associated with a plastidial origin, as may be concluded from the demonstration of melatonin in *Trypanosoma cruzi* (59), a nonphotosynthetic member of the Euglenozoa. Notably, the Excavata are phylogenetically distinct from the Archaeplastida.

Photosynthesis also occurs in the supergroup of the so-called SAR clade [Straminopiles (syn. Heterokonta), Alveolata, Rhizaria]. While most Rhizaria are heterotrophs, some of them, especially some giant foraminifers, take advantage from photosynthesis by either associating with phototroph dinoflagellates or by capturing chloroplasts ('kleptoplastids') from diatoms (60). Whether these foraminifers may be supplied with melatonin by their symbiotic dinoflagellates or from kleptoplastids is unknown. However, in the two other groups of the SAR clade, photosynthesis is frequently found. Among the Straminopiles, two large taxa are obligatory phototrophs, namely, the diatoms (Bacillariophyta) and the Phaeophyceae (brown algae), whereas two smaller taxa, the Labyrinthulea and the Oomycota (syn. Oomycetes) are nonphotosynthetic. While diatoms have apparently not yet been analyzed for the presence of melatonin, the brown alga *Pterygophora californica*, a giant kelp, was one of the first macroalgae in which melatonin was documented (61). Despite its presence in substantial amounts, melatonin did not show relevant variations within the day/night cycle. The major effect observed upon exogenous administration consisted in an inhibition of the growth rate. Although darkness also reduces growth, the action of melatonin may not be interpreted as transmission of the signal darkness. It may rather reflect something also known from Viridiplantae, namely an interference with the cytoskeleton, perhaps via calmodulin, a protein ubiquitously expressed in eukaryotes (62, 63). In the laboratory of K. Lüning, melatonin was also demonstrated in other brown algae, such as *Saccharina saccharina* (syn. *Laminaria saccharina*) (64), *Laminaria digitata*, and *Petalonia fascia* (1, 58), but in the latter cases, the information did not exceed the abstract level. Nevertheless, the general presence of melatonin in the Phaeophyceae seems highly likely.

Nonphotosynthetic Straminopiles have not been investigated with regard to melatonin formation. However, several species of the phytopathogen *Phytophthora* (Oomycota), formerly believed to be a fungus, but now known as members of a sister group of diatoms and brown algae, have been investigated under exposure of host plants to exogenous melatonin. The general outcome was an inhibition of pathogen development by melatonin (65, 66). This may be caused by a strengthening of the hosts' resistance. However, it also indicates that melatonin is presumably not produced at high concentrations by *Phytophthora*. Formation at low levels cannot be excluded, but the Oomycota cannot be taken as an outgroup argument for or against the general presence of melatonin in the Straminopiles.

The third SAR subclade, the Alveolata, contains many species that produce melatonin, sometimes in extremely high concentrations. In this regard, the subgroups, such as Chromerida, Apicomplexa (formerly known as Sporozoa), Ciliophora (syn. Ciliata), and Dinoflagellata, seem to differ. Only a few chromerid species are known, which are coral parasites. The chromerid *Chromera velia* that carries chloroplasts of rhodophycean origin, has not yet been investigated for the presence of melatonin. Chromerids seem to represent a sister group of the Apicomplexa, according to phylogenomic data. The Apicomplexa are also known to be parasites. Among them, the malaria pathogens of the *Plasmodium* genus are of particular interest beyond their medical importance, as their ancestors possessed chloroplasts before they evolved to parasites. A remnant of the former chloroplast exists as the nonphotosynthetic so-called apicoplast, which is surrounded by four membranes, indicating a secondary acquisition of the former chloroplast. This small organelle still participates in the metabolism of the parasite cell (67, 68). An apicoplast is also present in the less dangerous *Toxoplasma gondii*, but not in all Apicomplexa. Determinations of melatonin formation in Apicomplexa have not yet been carried out. However, effects of melatonin administration to hosts have been studied in several *Plasmodium* species. In some of them, such as *P. falciparum*, but not in all these species, melatonin was toxic to these parasites and entered

these cells from the circulation (69). Again, as in *Phytophthora*, the antiparasitic action of melatonin may indicate that this compound, if produced at all in these cells, cannot be formed in high concentrations.

In the third group of Apicomplexa, the ciliates (Ciliophora), melatonin has been repeatedly demonstrated. The ciliates comprise several mixotrophic species (e.g., *Labrea strobila*, *Strombidium viride*, several *Stentor* species), which contain either algal symbionts such as *Paramecium bursaria*, or captured chloroplasts (70, 71). Another ciliate, *Myrionecta rubra*, has been reported to be autotrophic (72). The presence of melatonin has not been documented in these species, but rather in several heterotrophs, such as *Tetrahymena thermophila* (30, 58), *Tetrahymena pyriformis* (73, 74), and *Eufolliculina* spec. (30). Additionally, the conversion of melatonin to AFMK was studied in *Paramecium caudatum* and *P. bursaria* (75). The formation of AFMK appeared to be rather specific in darkness, without any indication of a contribution of *Chlorella* symbionts present in *P. bursaria*. Generally, the capability of producing melatonin does not seem to depend on chloroplasts in ciliates, as judged from the few species studied. Interestingly, melatonin formation was stimulated in *Tetrahymena pyriformis* by pretreatment with melatonin and also by light (74). This lack of inhibition by light is reminiscent of findings in a phototrophic dinoflagellate, as will be described next. This may indicate that melatonin is not a mediator of darkness in alveolates, even not in those species that exhibit a nocturnal melatonin peak.

Dinoflagellates represent that alveolate group in which most information on melatonin is available. This molecule was first demonstrated in *Lingulodinium polyedrum* (at that time known under the name of *Gonyaulax polyedra*) (76). Thereafter, a high-amplitude circadian rhythm of melatonin with a nocturnal peak was described (77). This rhythm was shown to persist in constant darkness (78). In the course of subsequent studies, melatonin was demonstrated in numerous other phototrophic species, such as *Alexandrium lusitanicum*, *Ceratium horridum*, *Amphidinium carterae*, *Pyrocystis lunula*, and in the heterotrophic *Noctiluca scintillans*, as summarized elsewhere (1, 30, 58). It was also found in a *Symbiodinium* strain (79), a phototrophic symbiont of reef corals which can also exist as a free-swimming organism. In *Symbiodinium*, a nocturnally peaking rhythm was described, which, contrary to *L. polyedrum*, did not persist in constant darkness and was concluded not to be driven by a circadian oscillator. However, the melatonin rhythm in *Symbiodinium* exhibited a much lower amplitude than that of *Lingulodinium* and also did not show a unimodal pattern.

L. polyedrum is the dinoflagellate species in which most cell biological data are available on effects and metabolism of melatonin. Several of these findings can be generalized towards various related genera and species, but not to all dinoflagellates. *L. polyedrum* is a marine, phototrophic and bioluminescent species, which has been used as a chronobiological model organism (80). Direct effects of melatonin that were observed in this species concerned the suppression of diurnally peaking enzymes such as tryptophan hydroxylase and superoxide dismutase, modulation of the rhythm of glutathione S-transferase and, ecophysiologically important, the upregulation of aryl acylamidase (AAA) (58). A peculiarity of melatonin metabolism in this and related dinoflagellates concerns the deacetylation to 5-methoxytryptamine (5-MT) by AAA as the major catabolic pathway. 5-MT is further converted by a monoamine oxidase to 5-methoxyindole-3-acetaldehyde, which is metabolized to 5-methoxytryptophol (5-ML) and, in larger quantities, to 5-methoxyindole-3-acetic acid (5-MIAA). The latter metabolite is easily released from cells to the moderately alkaline seawater and represents melatonin's waste end product in *L. polyedrum* (62, 81). The deacetylation by AAA to 5-MT is of utmost importance to this species and other

dinoflagellates, too, since 5-MT is a potent inducer of asexual cysts, i.e., resting stages that survive adverse conditions (82). The signals that indicate adverse environment are lower temperature, short photoperiods and low availability of nitrate. Among them, low temperature seems to be the strongest signal. A decrease from rearing temperature of 20°C to 10 or 8°C induces encystment directly and rapidly, without any involvement of melatonin and 5-MT (83). However, such a strong drop is rather exceptionally experienced by a dinoflagellate, whereas a relatively moderate decrease to 15°C is possible in the course of nocturnal vertical migration, during which *Lingulodinium* cells descend by many meters down to nitrate-rich deeper water layers (84). However, a decrease to 15°C causes in *L. polyedrum* a dramatic increase in melatonin by orders of magnitude that exceeds by far the circadian variations. While the circadian maximum is in the range of 1 µM, the cold-induced increase leads to transient levels of several hundred µM or slightly above 1 mM (85, 86). This represents the by far strongest temperature effect on melatonin ever described. Notably, the temperature-induced increase was not prevented by light at rearing intensity. These high levels cause an upregulation of AAA, with the consequence of a rapid decrease of melatonin at the expense of rising 5-MT concentrations (85, 86). However, 5-MT is a strong inducer of encystment, with considerably higher efficiency than melatonin (82). These processes are counteracted by elevated nitrate concentrations and also by high preceding photosynthetic rates during the photophase (81). Therefore, *L. polyedrum* interprets adverse conditions that require an escape via encystment only if the lower temperature experienced during descending is not compensated by abundant nitrogen availability and opulent carbon fixation during the day. The melatonin metabolite 5-MT also regulates bioluminescence and seems to be required for the circadian glow peak at the end of the night, processes that do not require cyst-inducing levels and involve proton transfer from an acidic vacuole to the cytosolic side of the bioluminescent microsources, in *Lingulodinium* known as scintillons. A detailed description of the mechanisms is described elsewhere (87). Excessive proton transfer to the cytosol under the influence of 5-MT is also a key step in the course of encystment (7, 85, 86).

The question of whether the findings in *Lingulodinium* can be translated to other dinoflagellates requires differentiated answers. No encystment is possible in passively moving, coccoid dinoflagellates such as *Pyrocystis* species, as the coccoid wall represents a structure that is comparable to the cyst wall. Effects of melatonin and 5-MT on bioluminescence remained marginal. However, cyst induction by 5-MT has been observed in various actively swimming species, especially those which are closely related to *Lingulodinium*, such as several species or strains of *Alexandrium* (88). This was also reported for *Gymnodinium simplex*, *Aureodinium pigmentosum* (89) and *Crypthecodinium cohnii* (90, 91). However, the use of an automated procedure that only followed the sinking of immobilized cells may have been partially misleading, because identification of cysts requires microscopic examination. In fact, several species and strains (*Gymnodinium catenatum*; several *Prorocentrum* species) responded to 5-MT by immobilization, but not encystment, whereas others such as *Ceratium horridum* and *Amphidinium carterae* remained more or less insensitive to 5-MT (88). In some cases, cyst induction was also observed after addition of melatonin (58), but this should be most easily explained by conversion to 5-MT. A tropical, 5-MT-insensitive strain of *Amphidinium carterae* responded differently compared to *Lingulodinium* to temperature changes. In this case, a decrease from the rearing temperature of 24°C to 20°C caused reductions in melatonin, 5-MT and 5-ML, whereas an increase to 30°C led to strong augmentations of all three compounds (81).

A short comment seems to be due concerning some mechanistic claims in which effects of melatonin and 5-MT were attributed to calcium influx and several related signaling pathways as

well as binding of melatonin to a retinoid orphan receptor (RORs), which was concluded from effects of CGP 52608 (90, 91). This synthetic compound was previously believed to mimic melatonin effects via RORα. All these interpretations of signaling were problematic from several points of view (92): Some crucial effects were only observed at very high concentrations (1 or even 10 mM) that soon exceed the level of toxicity. The well-documented difference between cyst-inducing capacities of melatonin and 5-MT (82) did not appear in the calcium signaling experiments. Moreover, manipulation of calcium also influences the proton potential of the acidic vacuole membrane. Finally, the claim of nuclear effects via an ROR mechanism has to be dropped, mainly for two reasons: First, cyst induction starts much earlier than a transcriptional mechanism would allow. In bioluminescent species such as *L. polyedrum*, the beginning of the cyst-inducing effect can be easily traced on the basis of increased light emission which indicates proton transfer to the scintillon (87). This elevation is clearly present after less than 30 min of 5-MT exposure (1 or 100 µM) and almost immediately seen with its analog *N*,*N*-dimethyl-5-methoxytryptamine, which has a higher membrane permeability (93). Second, RORα has turned out to be incapable of binding melatonin (94, 95). As it has presumably evolved in early metazoans (96), it should be absent in dinoflagellates (97). Thus, the mechanistic interpretations mentioned in this paragraph have to be seen with caution and some of them have to be definitely dropped.

The exclusion of ROR signaling does not at all mean that melatonin has no transcriptional effects in dinoflagellates. Suppressive actions on diurnally peaking enzymes may well be explained by transcriptional control. The major problem results from the absence of identified melatonin receptors in this group of organisms. Tests for 2-[^{125}I]-iodomelatonin binding in sections from both day and night, as used in standard procedures for vertebrate G protein-coupled melatonin receptors, did not reveal positive results (98). Effects of the G protein targeting toxin mastoparan in *Crypthecodinium cohnii* (91) are not specific for melatonin or 5-MT. In the bioluminescent species *Gonyaulax spinifera*, long-term administration of mastoparan only moderately affected light emission, but did not yield 5-MT-like effects (99). Nevertheless, binding sites for melatonin may exist in *Lingulodinium*, as concluded from accumulation of exogenous melatonin. When 1 µM melatonin was added at dark onset to the medium, i.e., at the concentration of the endogenous circadian melatonin maximum, the intracellular level increased within three hours by up to 25-fold. When given at light onset, the accumulation was smaller, but still substantial (100). During cellular accumulation of melatonin, the medium concentration did not substantially change, because of the high medium/cell volume ratio. After three hours, the intracellular level dropped again, but remained higher than in the absence of exogenous melatonin. The concentration in the medium did not change during the intracellular decline. These findings indicate the existence of a melatonin-binding molecule, but this does not necessarily imply binding to a receptor and could also reflect another binding site not associated with a signaling pathway.

Concerning the role of melatonin in dinoflagellates, antioxidative protection has also been demonstrated, findings supporting the assumption that this represents one of the earliest functions of melatonin in evolution (7). Melatonin was shown to protect, at elevated but physiologically possible concentrations, *L. polyedrum* against H_2O_2 toxicity (101). Interestingly, the concentration of oxidatively damaged protein (protein carbonyl) strongly decreases during the last hours of photophase in an artificial light/dark cycle, i.e., in the phase when melatonin is already rising (102). Exposure to oxidotoxins such as paraquat and buthionine sulfoximine suppressed the circadian glow peak of *L. polyedrum*, but these effects were reversed by melatonin (103-105). Notably, melatonin did not upregulate antioxidant enzymes that are under melatonin control in vertebrates, such as superoxide dismutases, hemoperoxidases, glutathione peroxidase, or glutathione S-

transferase (101, 105). However, all oxidants mentioned strongly decreased the levels of melatonin and 5-MT (104, 105). The consumption of these antioxidants by radical-generating oxidotoxins may indicate that free radical scavenging plays a much more important role in *Lingulodinium* or, perhaps, dinoflagellates in general compared to vertebrates. This is not that much surprising, because the dinoflagellates studied contain considerably higher levels of melatonin which allow efficient detoxification at physiological concentrations. This may also be considered for other high-melatonin organisms, many of which are found, e.g., in Viridiplantae (81, 106).

4. MELATONIN IN ARCHAEPLASTIDA OUTSIDE THE VIRIDIPLANTAE

Contrary to the aforementioned taxa, the Archaeplastida represent the organisms that can be called plants *sensu stricto*. Information about melatonin in the two subclades of Archaeplastida that are different from Viridiplantae is relatively limited. In the small group of Glaucophyta, melatonin does not seem to have been investigated. More data are available for the Rhodophyceae (red algae). As summarized elsewhere (1, 58), the earlier studies detected melatonin in *Chondrus crispus*, *Gracilaria tenustipitata*, *Palmaria palmata*, and *Porphyra umbilicalis*. Presence of melatonin was later documented in *Kappaphycus alvarezii* (64) and *Pyropia yezoensis* (107). The absence of a circadian melatonin rhythm was reported for *Palmaria palmata* and *Kappaphycus alvarezii* (64). In *Porphyra umbilicalis*, the situation remained unclear, since a significant day/night difference was observed in one run, but did not reach significance in another one (64). Perhaps, comparisons of only day and night are not sufficient and a decision would require more densely taken time points.

More advanced investigations have been conducted much later (107). In *Pyropia yezoensis*, melatonin was not only determined, but its *Snat* gene was also cloned, the SNAT protein sequenced and compared to those of other organisms. Homology analyses showed clearly a closer relationship to the cyanobacterial enzyme than to those of land plants (pine and rice). Transfer of a *pySnat*-containing vector via *Agrobacterium* to tobacco leaves showed that the SNAT protein does not enter chloroplasts. Thus, the vector-transmitted protein is devoid of sorting peptides for plastidial localization, which is in agreement with the presence of the *pySnat* gene in the plastidial genome of the donor and, therefore, does not require protein transfer to this organelle in *P. yezoensis*.

The recombinant PySNAT enzyme exhibits a temperature dependence characterized by a strong increase towards 55°C, but inactivation by higher temperatures, whereas the enzymes from cyanobacteria and also from rice show increases up to 70°C. This difference should not be overrated, since temperatures above 55°C are presumably unbiological for marine species, even those which live in shallow water. In *Pyropia*, 25°C represent already heat stress. After 12 hours at 25°C, melatonin levels rose by about 50% (107). Whether melatonin conveys protection against heat stress in *Pyropia* or other rhodophyceans would require detailed studies.

5. MELATONIN IN LOWER VIRIDIPLANTAE

Chlorophyceae (green alga) represent the group from which land plants have evolved. Despite their diversity, unicellular and multicellular green algae as well as all types of land plants from bryophytes to tracheophytes collectively constitute the large monophyletic taxon of the Viridiplantae. The presence of melatonin was demonstrated in both unicellular and multicellular chlorophyceans. Among the unicells, melatonin was demonstrated in several organisms belonging to orders of different phylogenetic position. These reports concern *Dunaliella tertiolecta* (30, 58),

actually placed among the Chlamydomonales, sometimes also in a separated group, Duniellales, and several species and strains of *Chlamydomonas*, including *C. reinhardtii* (30, 58). In the latter species, a serotonin-converting *N*-acetylase had been cloned, which was described as crAANAT (108). However, the homology to other AANAT sequences was rather moderate. At that time, botanists frequently did not distinguish between SNAT and AANAT. The functional characterization confirmed the role as a serotonin acetylating enzyme, but the sequence indicates that it should be better regarded as a SNAT. The enzyme exhibited stronger homology to sequences from *Ostreococcus tauri* and *O. lucimarinus* (108), members of another chlorophycean order, the Mammiellales (syn. Micromonadales). With some likelihood, these organisms may also produce melatonin, which would, however, require direct confirmation, especially as sequences are not reliable indicators of substrate specificity.

Another melatonin producing species that lives in its vegetative phase as a giant unicell is *Acetabularia acetabulum* (30, 58), a member of the Dasycladales. In the vegetative cells, rhythmic changes in melatonin concentration were described, with a very narrow nocturnal peak that persisted in constant darkness, but was less prominent in constant light (30). When *Acetabularia* enters the generative phase, it forms a multicellular cap. In this phase, the melatonin rhythm was reported to be less expressed with average levels below those found in the vegetative life period (30). Notably, the Dasycladales to which *Acetabularia* belongs represent a subclade of the Ulvophyceae, in which other members are permanently multicellular. In the chlorophycean macroalga, *Ulva lactuca*, melatonin was reliably determined (64, 109). A day/night difference remained below statistical significance (64), but was not based on a sufficient number of time points for excluding rhythmicity. Another study in *Ulva* spec. (110) reported a low-amplitude rhythm with a significant maximum in the second half of scotophase. As *Ulva* lives in the tidal zone, the tidal influence may be more important than the light/dark cycle, as in many other organisms from such a biotope. This study also reported a correlation of melatonin with low tide (110). Moreover, melatonin was shown to be increased by heat and chemical (Zn, Pb, Cd) stress and to upregulate SOD activity (110). Thus, melatonin should be considered as a protective and antioxidant agent in *Ulva*. This may be in line with a medical report in which an ethanolic extract from *Ulva lactuca* was used for protective purposes against experimental myocardial infarction in rats (111). The extract should have contained melatonin, but its decisive relevance remains to be demonstrated. Nevertheless, *Ulva* may be seen as an example for a protective role of melatonin in primitive plants.

The Charales are another clade that is often regarded as green alga but is phylogenetically closer to the land plants, as it represents a sister group of the Embryophyta (land plants) within the clade of the Streptophyta. In a typical member of this group, *Chara australis*, melatonin was also detected (112). It was found in similar quantities in different regions of the organism, in photosynthetic tissue as well as in the chloroplast-free rhizoid, in vegetative and in reproductive parts of this plant. Addition of 10 µM melatonin to the water improved the quantum yield of photosystem II by 34%. The effect was interpreted in terms of an increase in the number of open reaction centers, which may be related to antioxidative protection of proteins involved in photosynthesis (112).

6. PRESUMABLE UBIQUITY OF MELATONIN IN LAND PLANTS

Concerning the basal clades of the Embryophyta, such as Marchantiophyta (liverworts), Bryophyta (mosses), Anthoceratophyta (horn mosses), and, among the Tracheophyta (vascular

plants), the Lycopodiophyta (club mosses and allies) and Polypodiopsida (ferns and allies), little is known about melatonin. In the moss *Physcomitrella patens* and in the lycopodiophyte *Selaginella moellendorfii* (a spike moss), SNAT homologs have been found (107). No relevant information exists for two basal gymnosperm clades of the Spermatophyta (seed plants), the Cycadopsida and the Gingkoopsida. However, in the third gymnosperm clade, the Coniferopsida (conifers and allies), substantial data have been obtained in *Pinus taeda* (113). In this species, a *Snat* gene was cloned and the amino acid sequence of the SNAT protein determined. This showed high homology to both the cyanophycean as well as to the angiosperm enzymes. This finding is strongly indicative for a conclusion that all streptophytes have inherited their plastids as well as their *Snat* genes from the same cyanophycean ancestor. However, this does not exclude an additional, mitochondrial source of serotonin acetylation, e.g., by an AANAT of α-proteobacterial origin (4). It should also not be excluded but rather expected that the proportion of both contributions to melatonin synthesis may vary between species. The PtSNAT localized to the chloroplasts, but it was only substantially expressed in the leaves, which correlated with high melatonin levels in only these plant organs (113).

In the angiosperms, the presence of melatonin is amply documented and has been repeatedly reviewed on the basis of comprehensive species lists (81, 106, 114, 115) or with focus on functional aspects (62, 63, 116-139). With regard to this large body of information, there is no need to repeat the numerous species in which melatonin has been found and the respective physiological details. However, it may be useful to have a look at the evolutionary basis of the numerous functions that melatonin has obviously acquired within the angiosperms.

A widely basal aspect of its functions in plants concerns its counteractions of stress, with frequent conveyance of stress resistance, as summarized in the reviews cited above. This includes protection against damage by heat, cold, drought, salt stress and heavy metal toxicity. All these functions may have evolved from the basic property as an antioxidant, since all these forms of stress are associated with oxidative damage. In the course of evolution, this function may have become extended from scavenging of free radicals to regulation of enzymatic mechanisms and transcriptional control of protective genes. Although the direct evidence for such an evolutionary process is missing, this assumption is highly suggestive, in particular, as the same situation is present in the phylogenetically distant vertebrates (140, 141). Similar considerations may be made with regard to anti-aging effects of melatonin, which have been especially studied in leaf senescence, perhaps also in the case of biotic stress by plant pathogens (63, 65, 66, 142-151).

The relationship to stress responses may also provide a hint towards the origin of melatonin's interactions with various phytohormones. Most of them are involved in the management of stress, as induced by different environmental challenges or pathogen infections. In various forms of stress, responses are, at least partially, similar and most frequently involve ethylene, abscisic, jasmonic and salicylic acids, sometimes also auxins, gibberellins, cytokinins, and brassinosteroids (152, 153). All of them have been shown to participate in drought stress responses (152). The use of melatonin by plants to combat damage by stress, in the beginning perhaps restricted to antioxidant actions, may have been an evolutionary driving force to promote interactions between melatonin and these other phytohormonal regulators. In the end, melatonin had attained a role in controlling numerous genes regulated by phytohormones (63, 129, 130, 132, 136, 154). Moreover, some phytohormones were shown to be modulated by melatonin (155). Having become a player within the phytohormonal network, it may have been only a short step for melatonin to also contribute to the control of growth, morphogenetic and fruit ripening processes. Of course, one cannot expect that these actions would be identical in all angiosperms.

The expectable deviations from species to species also appear likely with regard to the considerable differences of melatonin concentrations between organisms, plant organs and their derivatives such as seeds. The levels of melatonin vary from undetectable to, sometimes, several hundred µg/g (62, 106, 114). These variations indicate different roles of melatonin. Some of its functions that are based on hormone-like signaling may be more or less ubiquitous, whereas others requiring high melatonin levels may be restricted to those plants which are capable of producing elevated amounts. One of the functions based on high melatonin levels may be photoprotection. Interestingly, particularly high concentrations have been found in species or regional varieties that are exposed to high light and UV radiation, such as alpine, Mediterranean and fully light-exposed tropical plants (62, 63, 81, 114). In several plants, melatonin formation is light-inducible, which also indicates a photoprotective role. This may include a reduction of damage to photopigments and plastidial proteins by free radicals generated in the electron transport chains of the photosystems and by UV-induced singlet oxygen (62, 63). Under this perspective, the evolution toward high- or low-melatonin species does not reflect traits of particular clades, but rather an adaptation to certain habitats. Whether or not parasitic or saprophytic angiosperms that are free of chlorophyll, such as species of *Orobanche* and *Lathraea* (Orobanchaceae), *Monotropa* (Ericaceae), or *Neottia* (Orchidaceae), produce melatonin or take it up from their hosts, has not been studied. It might also be of interest to follow the relationship between photosynthesis and melatonin formation in *Ophrys speculum* (Orchidaceae), a species that can turn from a photosynthetic state via a partially bleached phase to a fully bleached, chlorophyll-lacking form.

7. PECULIARITIES OF MELATONIN METABOLISM IN PLANTS

Melatonin metabolism in Embryophyta has been reported to deviate in certain aspects from that known in Vertebrates. Generally, this can only concern melatonin biosynthesis and enzymatic catabolism. Nonenzymatic catabolism by free radicals or singlet oxygen should be, in principle, identical, although differences may exist in the relative proportions of the products (15). A major difference between plants and animals concerns the source of the precursor tryptophan. While animals have to take up this amino acid by the food, plants and all other phototrophs can synthesize tryptophan via the shikimic acid pathway. This allows much higher production rates of melatonin, since tryptophan availability is not limiting. The presence of the shikimic acid pathway should be considered as a primary, plesiomorphic property inherited from bacteria, particularly from cyanobacteria.

Concerning the conversion of tryptophan to serotonin, the main pathway in Viridiplantae (33) is different from that in animals and also in dinoflagellates (15). It consists of tryptophan decarboxylation by tryptophan decarboxylase (TDC), followed by tryptamine hydroxylation by tryptamine 5-hydroxylase (T5H). However, as in animals and dinoflagellates, the other route consisting of tryptophan hydroxylation by tryptophan hydroxylase (TPH) followed by decarboxylation of 5-hydroxytryptophan via an aromatic amino acid decarboxylase (AADC) or another decarboxylase (e.g., TDC?) seems to also exist in plants, because the intermediate 5-hydroxytryptophan has been demonstrated (33). The relevance of this second, animal-like pathway would require further clarification. In phylogenetic terms, the prevailing role of TDC in tryptophan conversion may be a trait that could be basal in Viridiplantae, although a definite judgment would require detailed studies in its unicellular members. Whether or not it is already a basal feature of Archaeplastida would need investigation in Rhodophyceae.

In Viridiplantae, the formation of melatonin from serotonin is possible via two routes. The first one consists of serotonin conversion to *N*-acetylserotonin by SNAT or, as far as expressed, AANAT, followed by *O*-methylation by ASMT (*N*-acetylserotonin *O*-methyltransferase) or COMT (caffeic acid *O*-methyltransferase). However, especially at high rates of melatonin formation, the alternate pathway (156) is also used by plants. It starts with *O*-methylation of serotonin by ASMT or COMT to 5-methoxytryptamine, followed by *N*-acetylation via SNAT (33). This alternate route is also present in other organisms (156) and, therefore, difficult to value from a phylogenetic point of view. Importantly, the situation in angiosperms is complicated by the existence of various isoenzymes in the melatonin biosynthetic pathways (33).

Concerning melatonin catabolism, all products known from nonenzymatic reactions of melatonin should occur in plants (15), whether already demonstrated or not. Among them, information is widely restricted to AFMK, which was demonstrated in rice, in which an indoleamine 2,3-dioxygenase (IDO) was identified and its gene cloned (157), and also in *Eichhornia crassipes* (158).

A major and quantitatively remarkable difference between the angiosperms studied and animals concerns the role of 2-hydroxylation as an enzymatic catabolic route. This reaction is catalyzed by melatonin 2-hydroxylase (M2H), a protein that belongs to the family of 2-oxoglutarate-dependent dioxygenases (2-ODDs) (159-162). In vertebrates, the product is also formed, e.g., by interaction with two hydroxyl radicals and under exposure to UVB (163) or by reacting with HOCl or its derivatives (15), but it does not attain high quantities as in plants, in which it often exceeds by far the levels of its parent compound melatonin, sometimes by a factor of more than 350-fold (161). This exceptional accumulation is explained by two causes, (i) the enzymatic formation that can involve several isoenzymes, and (ii) the fact that the primary product, 2-hydroxymelatonin, undergoes a tautomeric conversion to its keto tautomer, 2-acetamidoethyl-5-methoxyindolin- 2-one (15, 163, 165), also abbreviated as AMIO (15) (Fig. 1).

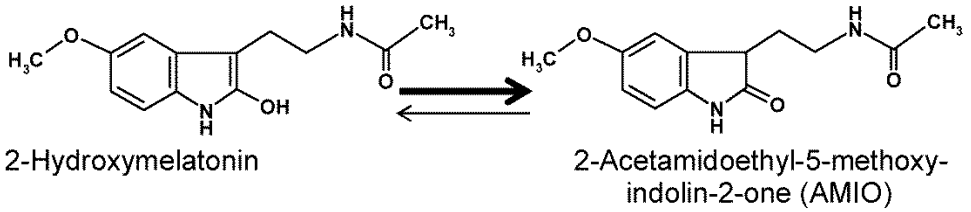

Fig. 1. The tautomeric equilibrium between 2-hydroxymelatonin and 2-acetamido-5-methoxyindolin-2-one is far on the side of the chemically rather inert keto compound.

The equilibrium is known since long to be far on the keto side (164, 165). AMIO was recently reported to attain close to 100% in the keto/enol mixture (166). Insofar, the real physiological product is almost completely AMIO rather than 2-hydroxymelatonin. Although the term 2-hydroxymelatonin has been used by many researchers, this is not only misleading because of the different structure, but even more because of the properties of AMIO, which are not really melatonin-like. In fact, AMIO is chemically much more inert than melatonin and its other hydroxylated derivatives (15, 166). Therefore, it is recommended to avoid the name 2-hydroxy-melatonin, which many readers and researchers would erroneously associate with melatonin's properties as a chemically reactive and potent antioxidant. The relative inertness and, thus, chemical stability of AMIO likely contributes to the strong accumulation of this compound by manifold above the melatonin level. Insofar, one should not expect antioxidant effects of AMIO

as far as they depend on chemical reactivity. However, this does not exclude other protective or even antioxidant actions if they are based on signaling mechanisms via receptor-like binding sites. For instance, this compound was reported to convey protection against abiotic stress in rice (167). In human colorectal cancer, it was also shown to exhibit antitumor activities (162). These effects in both plants and human should not be attributed to the minute traces of 2-hydroxymelatonin, but rather to the by far prevailing AMIO, and they will have to be explained by interactions with whichever binding sites. Although M2H is not known from animals, this enzyme may not be exclusively present in plants. It may have been inherited from bacteria, in which several 2-ODDs exist. Some 2-ODD isolates from E. coli were shown to hydroxylate melatonin in positions 3 or 2 (168).

8. CONCLUSION

As summarized in this article, melatonin is present in many clades of phototrophs (Table 1). In the case of angiosperms, it has been found in practically all species that were thoroughly investigated and can be considered to exist in all or nearly all of them. In most other groups of phototrophs, the number of positively tested species is considerably lower. As melatonin is already present in taxa that are basal to the embryophytes, such as various chlorophyceans and in a member of the Charales, it seems highly likely that it will be found in all other groups of embryophytes in which the demonstration is still lacking, as soon as their members are analyzed. The existence of melatonin in several rhodophyceans seems to indicate that it might be a general evolutionary character of all Archaeplastida, although the taxon of Glaucophyta has not yet been studied in this context. It would be of great interest to follow wether a Gα protein-coupled melatonin receptor that was recently described in Arabidopsis (169) is widely distributed in Viridiplantae and wether orthologs are found in other Archaeplastida. The possibility of additional receptor variants should be taken into consideration.

In the other clades of phototrophs that are not directly related to the Archaeplastida, the situation is often less clear. The presence of melatonin appears to be most certain in dinoflagellates, among which various species were studied in this regard and in which effects of the melatonin metabolite 5-MT were obtained in additional strains and species. Dinoflagellates represent outside the Viridiplantae the only group in which cell biological and ecophysiological mechanisms have been studied in detail. A possibly general presence of melatonin may be also assumed in brown algae, although the number of species studied is still limited. Moreover, lacking information on their large sister group, the diatoms, is perceived as regrettable gap. A major difference to the Archaeplastida concerns the fact that the phototrophs within the Excavata and the SAR clade are secondary phototrophs (meta-algae) and that the respective taxa often contain both photo- and heteroptrophs. This is the case in Euglenozoa and in Alveolata. In both clades, some heterotrophs were also found to produce melatonin. However, it remains uncertain whether this is valid for all of them. If melatonin will be found in many more primary heterotrophic species from these clades, this would be indicative of a general, plesiomorphic capability to synthesize this molecule in eukaryotes, a property that should have been inherited from α-proteobacteria, provided that the genes of the biosynthetic enzymes have not been taken up by horizontal gene transfer.

The evolutionary fate of melatonin within eukaryotes and, particularly, in their phototrophs is more complicated than usually perceived, especially as the progressing elaboration of the phylogenetic tree has revealed many new dichotomies and connections. The considerable gaps that are evident in Table 1 may hopefully encourage investigators to fill them.

Table 1. Presence of melatonin in eukaryotic clades with phototrophic members and a few sister groups.

Taxon	**Melatonin (Mel) in 4 trophic categories #**			
	H1	**P1**	**P2**	**H2**
Excavata				
Euglenozoa	Mel		Mel	
SAR clade				
Straminopiles				
Bacillariophyta			n.d.	
Phaeophyceae			Mel	
Labyrinthulea	n.d.			
Oomycota				n.d.†
Alveolata				
Chromerida			n.d.	
Apicomplexa				n.d.†
Ciliophora	Mel		n.d.	
Dinoflagellata	Mel		Mel	
Rhizaria	n.d.		n.d.	
Archaeplastida				
Glaucophyta		n.d.		
Rhodophyceae		Mel		
Viridiplantae				
Chlorophyceae				
Chlamydomonales		Mel		
Mammiellales		Mel?		
Ulvophyceae				
Dasycladales		Mel		
Ulvales		Mel		
Streptophyta				
Charales		Mel		
Embryophyta				
Marchantiophyta		n.d.		
Bryophyta		Mel?		
Anthoceratophyta		n.d.		
Tracheophyta				
Lycopodiophyta		Mel		
Polypodiopsida		n.d.		
Spermatophyta				
Gymnospermae				
Cycadopsida		n.d.		
Gingkoopsida		n.d.		
Coniferopsida		Mel		
Angiospermae		Mel		n.d.*

*H1 – primary heterotrophs; P1 – primary phototrophs by association with cyanobacterial endosymbionts in early evolution; P2 – secondary phototrophs by association with chloroplast-containing eukaryotes or by chloroplast capture; H2 – secondary heterotrophs by anatomical or functional loss of chloroplasts. n.d.: not determined. *Only very few species. † Parasites which are inhibited by high exogenous melatonin. Mel? refers to species in which SNAT homologs were found, but melatonin was not determined.*

ACKNOWLEDGMENT

None. The manuscript did not require financial support.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. Hardeland R, Fuhrberg B (1996) Ubiquitous melatonin – Presence and effects in unicells, plants and animals. *Trends Comp. Biochem. Physiol.* **2**: 25-45.
2. Hardeland R, Poeggeler B (2003) Non-vertebrate melatonin. *J. Pineal Res.* **34**: 233-241. doi: 10.1034/j.1600-079X.2003.00040.x.
3. Tan, D-X, Hardeland R, Manchester LC, *et al.* (2010) The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. *Biol. Rev. Camb. Philos. Soc.* **85**: 607-623. doi: 10.1111/j.1469-185X.2009.00118.x.
4. Tan D-X, Reiter RJ (2019) Mitochondria: the birth place, battle ground and site of melatonin metabolism in cells. *Melatonin Res*. **2**: 44-66. doi: 10.32794/mr11250011.
5. Biarrotte-Sorin S, Mayer C (2005) Cloning, purification, crystallization and preliminary crystallographic analysis of a hypothetical acetyltransferase from *Pyrococcus furiosus*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun*. **61** (Pt 3): 269-270. doi: 10.1107/S174430910500223X.
6. Ma C, Pathak C, Jang S, *et al.* (2014) Structure of *Thermoplasma volcanium* Ard1 belongs to N-acetyltransferase family member suggesting multiple ligand binding modes with acetyl coenzyme A and coenzyme A. *Biochim. Biophys. Acta* **1844**: 1790-1797. doi: 10.1016/j.bbapap.2014.07.011.
7. Hardeland R, Balzer I, Poeggeler B, *et al.* (1995) On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. *J. Pineal Res*. **18**: 104-111. doi: 10.1111/j.1600-079X.1995.tb00147.x.
8. Tan D-X, Chen L-D, Poeggeler B, *et al.* (1993) Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocr. J*. **1**: 57-60.
9. Tan D-X, Reiter RJ, Manchester LC, *et al.* (2002) Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Curr. Top. Med. Chem*. **2**: 181-197. doi: 10.2174/1568026023394443.
10. Hardeland R, Pandi-Perumal SR, Cardinali DP (2006) Melatonin. *Int. J. Biochem. Cell Biol*. **38**: 313-316. doi: 10.1016/j.biocel.2005.08.020.
11. Pandi-Perumal SR, Srinivasan V, Maestroni GJM, *et al.* (2006) Melatonin – Nature's most versatile biological signal? *FEBS J*. **273**: 2813-2838. doi: 10.1111/j.1742-4658.2006.05322.x.

12. Poeggeler B, Reiter RJ, Tan D-X, *et al.* (1993) Melatonin, hydroxyl radical-mediated oxidative damage, and aging: a hypothesis. *J. Pineal Res*. **14**: 151-168. doi: 10.1111/j.1600-079X.1993.tb00498.x.
13. Hardeland R, Reiter RJ, Poeggeler B, *et al.* (1993) The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. *Neurosci. Biobehav. Rev.* **17**: 347-357. doi: 10.1016/S0149-7634(05)80016-8.
14. Poeggeler B, Thuermann S, Dose A, *et al.* (2002) Melatonin's unique radical scavenging properties - roles of its functional substituents as revealed by a comparison with its structural analogs. *J. Pineal Res*. **33**: 20-30. doi: 10.1034/j.1600-079X.2002.01873.x.
15. Hardeland R (2017) Taxon- and site-specific melatonin catabolism. *Molecules* **22**: E2015. doi: 10.3390/molecules22112015.
16. Tan D-X, Manchester LC, Reiter RJ, *et al.* (1998) A novel melatonin metabolite, cyclic 3-hydroxymelatonin: a biomarker of in vivo hydroxyl radical generation. *Biochem. Biophys. Res. Commun*. **253**: 614-620. doi: 10.1006/bbrc.1998.9826.
17. Tan D-X, Manchester LC, Reiter RJ, *et al.* (1999) Cyclic 3-hydroxymelatonin: a melatonin metabolite generated as a result of hydroxyl radical scavenging. *Biol. Signals Recept*. **8**: 70-74. doi: 10.1159/000014571.
18. Hardeland R, Tan D-X, Reiter RJ (2009) Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. *J. Pineal Res*. **47**: 109-126. doi: 10.1111/j.1600-079X.2009.00701.x.
19. Tan D-X, Hardeland R, Manchester LC, *et al.* (2014) Cyclic-3-hydroxymelatonin (C3HOM), a potent antioxidant, scavenges free radicals and suppresses oxidative reactions. *Curr. Med. Chem.* **21**: 1557-1565. doi: 10.2174/0929867321666131129113146.
20. Burkhardt S, Reiter RJ, Tan D-X, *et al.* (2001) DNA oxidatively damaged by chromium(III) and H_2O_2 is protected by the antioxidants melatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine, resveratrol and uric acid. *Int. J. Biochem. Cell Biol*. **33**: 775-783. doi: 10.1016/S1357-2725(01)00052-8.
21. Tan D-X, Manchester LC, Burkhardt S, *et al* (2001) N^1-acetyl-N^2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. *FASEB J*. **15**: 2294-2296. doi: 10.1096/fj.01-0309fje.
22. Rosen J, Than NN, Koch D, *et al.* (2006) Interactions of melatonin and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. *J. Pineal Res*. **41**: 374-381. doi: 10.1111/j.1600-079X.2006.00379.x.
23. de Almeida EA. Martinez GR, Klitzke CF, *et al.* (2003) Oxidation of melatonin by singlet molecular oxygen ($O_2(^1\Delta_g)$) produces N^1-acetyl-N^2-formyl-5-methoxykynurenine. *J. Pineal Res*. **35**: 131-137. doi: 10.1034/j.1600-079X.2003.00066.x.
24. Hardeland R, Poeggeler B, Balzer I, *et al.* (1993) A hypothesis on the evolutionary origins of photoperiodism based on circadian rhythmicity of melatonin in phylogenetically distant organisms. In: *Chronobiology & Chronomedicine* (Gutenbrunner C, Hildebrandt G, Moog R, eds.), Lang, Frankfurt/M. - Berlin - Bern - New York - Paris - Vienna, pp. 113-120.
25. Behrmann G, Fuhrberg B, Hardeland R, *et al.* (1997) Photooxidation of melatonin, 5-methoxytryptamine and 5-methoxytryptophol: aspects of photoprotection by periodically fluctuating molecules? *Biometeorology* 14, Pt. 2/2: 258-263.

26. Schaefer M, Hardeland R (2009) The melatonin metabolite N^1-acetyl-5-methoxykynuramine is a potent singlet oxygen scavenger. *J Pineal Res*. **46**: 49-52. doi: 10.1111/j.1600-079X.2008.00614.x.
27. Hardeland R, Poeggeler B, Balzer I, *et al.* (1991) Common basis of photoperiodism in phylogenetically distant organisms and its possible origins. *J. Interdiscipl. Cycle Res*. **22**: 122-123. doi: 10.1080/09291019109360102.
28. Manchester LC, Poeggeler B, Alvares FL, *et al.* (1995) Melatonin immunoreactivity in the photosynthetic prokaryote *Rhodospirillum rubrum*: Implications for an ancient antioxidant system. *Cell. Mol. Biol. Res*. **41**: 391-395.
29. Tilden AR, Becker MA, Amma LL, *et al.* (1997) Melatonin production in an aerobic photosynthetic bacterium: an evolutionarily early association with darkness. *J. Pineal Res*. **22**: 102-106. doi: 10.1111/j.1600-079X.1997.tb00310.x
30. Balzer I, Höcker B, Kapp H, *et al.* (2000) Occurrence and comparative physiology of melatonin in evolutionary diverse organisms. In: *The Redox State and Circadian Rhythms* (Vanden Driessche T, Guisset J-L, Pertieau-de Vries, GM, eds.), Kluwer, Dordrecht – Boston – London, pp. 95-119.
31. Hattori A, Wada M, Majima A, *et al*. (1999) Detection of melatonin and synthesizing enzyme activities in cyanobacterium, *Spirulina platensis*. *Proc. Jpn. Soc. Comp. Endocrinol*. **14**: 49.
32. Majima A, Hattori A, Wada M, *et al.* (1999) Dynamic release of melatonin in cyanobacterium, *Spirulina platensis*. *Proc. Jpn. Soc. Comp. Endocrinol.* **14**: 50.
33. Back K, Tan D-X, Reiter RJ (2016) Melatonin biosynthesis in plants: multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts. *J. Pineal Res*. **61**: 426-437. doi: 10.1111/jpi.12364.
34. Byeon Y, Lee K, Park Y-I, *et al.* (2013) Molecular cloning and functional analysis of serotonin *N*-acetyltransferase from the cyanobacterium *Synechocystis* sp. PCC 6803. *J. Pineal Res*. **55**: 371-376. doi: 10.1111/jpi.12080.
35. Byeon Y, Lee HY, Lee K, *et al.* (2014) Cellular localization and kinetics of the rice melatonin biosynthetic enzymes SNAT and ASMT. *J. Pineal Res*. **56**: 107-114. doi: 10.1111/jpi.12103.
36. Wang L, Feng C, Zheng X, *et al* (2017) Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. *J. Pineal Res*. **63**: e12429. doi: 10.1111/jpi.12429.
37. Tan D-X, Manchester LC, Liu X, *et al.* (2013) Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *J. Pineal Res*. **54**: 127-138. doi: 10.1111/jpi.12026.
38. Tan D-X, Zheng X, Kong J, *et al.* (2014) Fundamental issues related to the origin of melatonin and melatonin isomers during evolution: relation to their biological functions. *Int. J. Mol. Sci*. **15**: 15858-15890. doi: 10.3390/ijms150915858.
39. Coon SL, Klein DC (2006) Evolution of arylalkylamine *N*-acetyltransferase: emergence and divergence. *Mol. Cell. Endocrinol*. **252**: 2-10. doi: 10.1016/j.mce.2006.03.039.
40. Suofu Y, Li W, Jean-Alphonse FG, *et al.* (2017) Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc. Natl. Acad. Sci. USA* **114**: E7997-E8006. doi: 10.1073/pnas.1705768114.
41. Abhishek A, Bavishi A, Bavishi A, *et al.* (2011) Bacterial genome chimaerism and the origin of mitochondria. *Can. J. Microbiol*. **57**: 49-61. doi: 10.1139/w10-099.
42. Torrens-Spence MP, Liu P, Ding H, *et al.* (2013) Biochemical evaluation of the decarboxylation and decarboxylation-deamination activities of plant aromatic amino acid decarboxylases. *J. Biol. Chem*. **288**: 2376-2387. doi: 10.1074/jbc.M112.401752.

43. Han Q, Robinson H, Ding H, *et al.* (2012) Evolution of *N*-acetyltransferases: structural evidence from the yellow fever mosquito, *Aedes aegypti*. *Proc. Natl. Acad. Sci. USA* **109**: 11669-11674. doi: 10.1073/pnas.1206828109.
44. Hiragaki S, Suzuki T, Mohamed AA, *et al.* (2015) Structures and functions of insect arylalkylamine *N*-acetyltransferase (iaaNAT), a key enzyme for physiological and behavioral switch in arthropods. *Front. Physiol*. **6**: 113. doi: 10.3389/fphys.2015.00113.
45. Slominski A, Fischer TW, Zmijewski MA, *et al.* (2005) On the role of melatonin in skin physiology and pathology. *Endocrine* **27**: 137-148. doi: 10.1385/ENDO:27:2:137.
46. Dang H, Lovell CR (2015) Microbial surface colonization and biofilm development in marine environments. *Microbiol. Mol. Biol. Rev*. **80**: 91-138. doi: 10.1128/MMBR.00037-15.
47. Kamekura M (1998) Diversity of extremely halophilic bacteria. *Extremophiles* **2**: 289-295.
48. Oren A (2014) Taxonomy of halophilic Archaea: current status and future challenges. *Extremophiles* **18**: 825-834. doi: 10.1007/s00792-014-0654-9.
49. Spang A, Stairs CW, Dombrowski N, *et al.* (2019) Proposal of the reverse flow model for the origin of the eukaryotic cell based on comparative analyses of Asgard archaeal metabolism. *Nat. Microbiol*. [Epub ahead of print, Apr 1]. doi: 10.1038/s41564-019-0406-9.
50. Schwartzbach SD, Osafune T, Löffelhardt W (1998) Protein import into cyanelles and complex chloroplasts. *Plant Mol. Biol*. **38**: 247-263.
51. Cavalier-Smith T (2003) Genomic reduction and evolution of novel genetic membranes and protein-targeting machinery in eukaryote-eukaryote chimaeras (meta-algae). *Philos. Trans. R. Soc. Lond. B Biol. Sci*. **358**: 109-133. doi: 10.1098/rstb.2002.1194.
52. Figueroa RI, Bravo I, Fraga S, *et al.* (2009) The life history and cell cycle of *Kryptoperidinium foliaceum*, a dinoflagellate with two eukaryotic nuclei. *Protist* **160**: 285-300. doi: 10.1016/j.protis.2008.12.003.
53. Archibald JM (2009) The puzzle of plastid evolution. *Curr. Biol*. **19**: R81-R88. doi: 10.1016/j.cub.2008.11.067.
54. Moore RB, Oborník M, Janouškovec J, *et al.* (2008) A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* **451**: 959-963. doi: 10.1038/nature06635.
55. Nowack ECM, Melkonian M, Glöckner G (2008) Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr. Biol*. **18**: 410-418. doi: 10.1016/j.cub.2008.02.051.
56. Prechtl J, Kneip C, Lockhart P, *et al.* (2004) Intracellular spheroid bodies of *Rhopalodia gibb*a have nitrogen-fixing apparatus of cyanobacterial origin. *Mol. Biol. Evol*. **21**: 1477-1481. doi: 10.1093/molbev/msh086.
57. Balzer I, Fuhrberg B, Hardeland R (1996) The neurohormone melatonin oscillates in a circadian fashion already in unicells. In: *Brain and Evolution* (Elsner N., Schnitzler H-U, eds.), Thieme, Stuttgart - New York, p. 228.
58. Hardeland R (1999) Melatonin and 5-methoxytryptamine in non-metazoans. *Reprod. Nutr. Dev*. **39**: 399-408. doi: 10.1051/rnd:19990311.
59. Macías M, Rodríguez-Cabezas MN, Reiter RJ, *et al.* (1999) Presence and effects of melatonin in *Trypanosoma cruzi*. *J. Pineal Res*. **27**: 86-94. doi: 10.1111/j.1600-079X.1999.tb00601.x.
60. Bernhard JM, Bowser SS (1999) Benthic foraminifera of dysoxic sediments: chloroplast sequestration and functional morphology. *Earth Sci. Rev*. **46**: 149-165. doi: 10.1016/S0012-8252(99)00017-3.

61. Fuhrberg B, Balzer I, Hardeland R, *et al.* (1996) The vertebrate pineal hormone melatonin is produced by the brown alga *Pterygophora californica* and mimics dark effects on growth rate in the light. *Planta* **200**: 125-131. doi: 10.1007/BF00196659.
62. Hardeland R (2015) Melatonin in plants and other phototrophs – advances and gaps concerning the diversity of functions. *J. Exp. Bot*. **66**: 627-646. doi: 10.1093/jxb/eru386.
63. Hardeland R (2016) Melatonin in plants - diversity of levels and multiplicity of functions. *Front. Plant Sci*. **7**: 198. doi: 10.3389/fpls.2016.00198.
64. Pape C (2004) Melatoningehalt in marinen Makroalgen. Entwicklung und Validierung quantitativer Bestimmungen mittels HPLC und Enzym-gekoppeltem Immunoassay. *Ber. Polar Meeresforsch*. **473**. doi: 10.2312/BzPM_0473_2004.
65. Zhang S, Zheng X, Reiter RJ, *et al.* (2017) Melatonin attenuates potato late blight by disrupting cell growth, stress tolerance, fungicide susceptibility and homeostasis of gene expression in *Phytophthora infestans*. *Front. Plant Sci*. **8**: 1993. doi: 10.3389/fpls.2017.01993.
66. Zhang S, Liu S, Zhang J, *et al.* (2018) Synergistic anti-oomycete effect of melatonin with a biofungicide against oomycetic black shank disease. *J. Pineal Res*. **65**: e12492. doi: 10.1111/jpi.12492.
67. Botté CY, Yamaryo-Botté Y (2018) Complex endosymbioses II: The nonphotosynthetic plastid of Apicomplexa parasites (the apicoplast) and its integrated metabolism. *Methods Mol. Biol.* **1829**: 37-54. doi: 10.1007/978-1-4939-8654-5_3.
68. Kadian K, Gupta Y, Singh HV, *et al.* (2018) Apicoplast metabolism: Parasite's Achilles' heel. *Curr. Top. Med. Chem*. **18**: 1987-1997. doi: 10.2174/1568026619666181130134742.
69. Bagnaresi P, Nakabashi M, Thomas AP, *et al* (2012) The role of melatonin in parasite biology. *Mol. Biochem. Parasitol*. **181**: 1-6. doi: 10.1016/j.molbiopara.2011.09.010.
70. Putt M (1990) Abundance, chlorophyll content and photosynthetic rates of ciliates in the Nordic Seas during summer. *Deep Sea Res. A Oceanogr. Res*. **37**: 1713-1731. doi: 10.1016/0198-0149(90)90073-5.
71. Laybourn-Parry J, Perriss SJ, Seaton GGR, *et al.* (1997) A mixotrophic ciliate as a major contributor to plankton photosynthesis in Australian lakes. *Limnol. Oceanogr*. **42**: 1463-1467.
72. Sanders RW (1995) Seasonal distributions of the photosynthesizing ciliates *Laboea strobila* and *Myrionecta rubra* (= *Mesodinium rubrum*) in an estuary of the Gulf of Maine. *Aquat. Microb. Ecol*. **9**: 237-242.
73. Köhidai L, Vakkuri O, Keresztesi M, *et al.* (2002) Melatonin in the unicellular *Tetrahymena pyriformis*: effects of different lighting conditions. *Cell Biochem. Funct*. **20**: 269-272.
74. Köhidai L, Vakkuri O, Keresztesi M, *et al.* (2003) Induction of melatonin synthesis in *Tetrahymena pyriformis* by hormonal imprinting--a unicellular "factory" of the indoleamine. *Cell. Mol. Biol. (Noisy-le-grand)* **49**: 521-524.
75. Poeggeler B, Hardeland R (2001) Observations on melatonin oxidation and metabolite release by unicellular organisms and small aquatic metazoans. In: *Actions and Redox Properties of Melatonin and Other Aromatic Amino Acid Metabolites* (Hardeland R, ed.), Cuvillier, Göttingen, pp. 66-69.
76. Poeggeler B, Balzer I, Fischer J, *et al.* (1989) A role of melatonin in dinoflagellates? *Acta Endocrinol. (Cop.)* **120**: Suppl. 1: 97.
77. Poeggeler B, Balzer I, Hardeland R, *et al.* (1991) Pineal hormone melatonin oscillates also in the dinoflagellate *Gonyaulax polyedra*. *Naturwissenschaften* **78**: 268-269.
78. Balzer I, Poeggeler B, Hardeland R (1993) Circadian rhythms of indoleamines in a dinoflagellate, *Gonyaulax polyedra*: Persistence of melatonin rhythm in constant darkness and

relationship to 5-methoxytryptamine. In: *Melatonin and the Pineal Gland - From Basic Science to Clinical Application* (Touitou Y, Arendt J, Pévet P, eds.), Excerpta Medica, Amsterdam - London – New York - Tokyo, pp. 183-186.
79. Roopin M, Yacobi YZ, Levy O (2013) Occurrence, diel patterns, and the influence of melatonin on the photosynthetic performance of cultured *Symbiodinium*. *J. Pineal Res*. **55**: 89-100. doi: 10.1111/jpi.12046.
80. Dunlap JC, Hastings JW (1981) The biological clock in *Gonyaulax* controls luciferase activity by regulating turnover. *J. Biol. Chem*. **256**: 10509-10518.
81. Hardeland R, Pandi-Perumal SR, Poeggeler B (2007) Melatonin in plants – Focus on a vertebrate night hormone with cytoprotective properties. *Funct. Plant Sci. Biotechnol*. **1**: 32-45.
82. Balzer I, Hardeland R (1991) Photoperiodism and effects of indoleamines in a unicellular alga, *Gonyaulax polyedra*. *Science* **253**: 795-797. doi: 10.1126/science.1876838.
83. Hardeland R (1994) Induction of cyst formation by low temperature in the dinoflagellate *Gonyaulax polyedra* Stein: dependence on circadian phase and requirement of light. *Experientia* **50**: 60-62.
84. Roenneberg T, Rehman J (1996) Nitrate, a nonphotic signal for the circadian system. *FASEB J*. **10**: 1443-1447. doi: 10.1096/fasebj.10.12.8903515.
85. Fuhrberg B, Hardeland R, Poeggeler B, *et al.* (1997) Dramatic rises of melatonin and 5-methoxytryptamine in *Gonyaulax* exposed to decreased temperature. *Biol. Rhythm Res*. **28**: 144-150. doi: 10.1076/brhm.28.1.144.12978.
86. Fuhrberg B, Hardeland R (1997) Temperature as a major environmental factor controlling levels and rhythm amplitudes of melatonin in the marine dinoflagellate *Gonyaulax polyedra*. *Biometeorology* **14**, Pt. 2/2: 272-277.
87. Hardeland R, Hoppenrath M (2012) Bioluminescence in dinoflagellates. *Tree of Life*; http://tolweb.org/articles/?article_id=5621.
88. Hardeland R, Mbachu EM, Fuhrberg B (1999) Asexual cyst induction in dinoflagellates: Differences in encystment competence do not generally correspond with responsiveness to 5-methoxytryptamine. In: *Studies on Antioxidants and their Metabolites* (Hardeland R, ed.), Cuvillier, Göttingen, pp. 177-183.
89. Wong JTY, Wong YH (1994) Indoleamine-induced encystment in dinoflagellates. *J. Mar. Biol. Assoc. U.K*. **74**: 467-469. doi: 10.1017/S0025315400039515.
90. Tsim ST, Wong JT, Wong YH (1997) Calcium ion dependency and the role of inositol phosphates in melatonin-induced encystment of dinoflagellates. *J. Cell Sci*. **110**: 1387-1393.
91. Tsim ST, Wong JT, Wong YH (1998) Regulation of calcium influx and phospholipase C activity by indoleamines in dinoflagellate *Crypthecodinium cohnii*. *J. Pineal Res*. **24**: 152-161. doi: 10.1111/j.1600-079X.1998.tb00528.x.
92. Hardeland R (1999) Indoleamine-induced encystment in dinoflagellates: On the problems of distinguishing between proton and calcium effects. In: *Studies on Antioxidants and their Metabolites* (Hardeland R, ed.), Cuvillier, Göttingen, pp. 184-190.
93. Balzer I, Hardeland R (1991) Stimulation of bioluminescence by 5-methoxylated indoleamines in the dinoflagellate, *Gonyaulax polyedra*. *Comp. Biochem. Physiol*. **98 C**: 395-397.
94. Slominski AT, Kim TK, Takeda Y, *et al.* (2014) RORα and RORγ are expressed in human skin and serve as receptors for endogenously produced noncalcemic 20-hydroxy- and 20,23-dihydroxyvitamin D. *FASEB J*. **28**: 2775-2789. doi: 10.1096/fj.13-242040.

95. Slominski AT, Zmijewski MA, Jetten AM (2016) RORα is not a receptor for melatonin (response to DOI 10.1002/bies.201600018). *Bioessays* **38**: 1193-1194. doi: 10.1002/bies.201600204.
96. Owen GI, Zelent A (2000) Origins and evolutionary diversification of the nuclear receptor superfamily. *Cell. Mol. Life Sci*. **57**: 809-827. doi: 10.1007/s000180050043.
97. Hardeland R (2018) Melatonin and retinoid orphan receptors: Demand for new interpretations after their exclusion as nuclear melatonin receptors. *Melatonin Res*. **1**: 77-92. doi: 10.32794/mr11250005.
98. Masson-Pévet M, Balzer I, Hardeland R (1997) Testing for melatonin receptors in *Gonyaulax polyedra*. In: *Biological Rhythms and Antioxidative Protection* (Hardeland R, ed.), Cuvillier, Göttingen, pp. 107-109.
99. Mbachu EM, Hardeland R (1999) Effects of mastoparan on bioluminescence in *Gonyaulax spinifera*. In: *Studies on Antioxidants and their Metabolites* (Hardeland R, ed.), Cuvillier, Göttingen, pp. 172-176.
100. Mueller U, Hardeland R (1999) Transient accumulations of exogenous melatonin indicate binding sites in the dinoflagellate *Gonyaulax polyedra*. In: *Studies on Antioxidants and their Metabolites* (Hardeland R, ed.), Cuvillier, Göttingen, pp. 140-147.
101. Antolín I, Obst B, Burkhardt S, *et al* (1997) Antioxidative protection in a high-melatonin organism: The dinoflagellate *Gonyaulax polyedra* is rescued from lethal oxidative stress by strongly elevated, but physiologically possible concentrations of melatonin. *J. Pineal Res*. **23**: 182-190. doi: 10.1111/j.1600-079X.1997.tb00353.x.
102. Hardeland R, Coto-Montes A, Burkhardt S, *et al.* (2000) Circadian rhythms and oxidative stress in non-vertebrate organisms. In: *The Redox State and Circadian Rhythms* (Vanden Driessche T, Guisset J-L, Petiau-de Vries G, eds.), Kluwer, Dordrecht - Boston - London, pp. 121-140.
103. Antolín I, Hardeland R (1997) Suppression of the *Gonyaulax* glow peak by paraquat and its restoration by melatonin. In: *Biological Rhythms and Antioxidative Protection* (Hardeland R, ed.), Cuvillier, Göttingen, pp. 86-97.
104. Hardeland R, Burkhardt S, Antolín I, *et al.* (1999) Melatonin and 5-methoxytryptamine in the bioluminescent dinoflagellate *Gonyaulax polyedra*: Restoration of the circadian glow peak after suppression of indoleamine biosynthesis or oxidative stress. *Adv. Exp. Med. Biol*. **460**: 387-390.
105. Hardeland R, Coto-Montes A (2000) Chronobiology of oxidative stress and anti-oxidative defense mechanisms. In: *Recent Research Developments in Comparative Biochemistry and Physiology*, Vol. 1 (Pandalai SG, ed.), Transworld Research Network, Trivandrum, pp. 123-137.
106. Chen G, Huo Y, Tan D-X, *et al.* (2003) Melatonin in Chinese medicinal herbs. *Life Sci*. **73**: 19-26. doi: 10.1016/S0024-3205(03)00252-2.
107. Byeon Y, Yool Lee H, Choi DW, *et al.* (2015) Chloroplast-encoded serotonin *N*-acetyltransferase in the red alga *Pyropia yezoensis*: gene transition to the nucleus from chloroplasts. *J. Exp. Bot*. **66**: 709-717. doi: 10.1093/jxb/eru357.
108. Okazaki M, Higuchi K, Hanawa Y, *et al.* (2009) Cloning and characterization of a *Chlamydomonas reinhardtii* cDNA arylalkylamine *N*-acetyltransferase and its use in the genetic engineering of melatonin content in the Micro-Tom tomato. *J. Pineal Res*. **46**: 373-82. doi: 10.1111/j.1600-079X.2009.00673.x.

109. Pape C, Lüning K (2006) Quantification of melatonin in phototrophic organisms. *J. Pineal Res*. **41**: 157-165. doi: 10.1111/j.1600-079X.2006.00348.x.
110. Tal O, Haim A, Harel O, *et al.* (2011) Melatonin as an antioxidant and its semi-lunar rhythm in green macroalga *Ulva* sp. *J. Exp. Bot*. **62**: 1903-1910. doi: 10.1093/jxb/erq378.
111. Widyaningsih W, Pramono S, Zulaela, *et al.* (2017) Protection by ethanolic extract from *Ulva lactuca* L. against acute myocardial infarction: antioxidant and antiapoptotic activities. *Malays. J. Med. Sci*. **24**: 39-49. doi: 10.21315/mjms2017.24.6.5.
112. Lazár D, Murch SJ, Beilby MJ, *et al.* (2013) Exogenous melatonin affects photosynthesis in characeae *Chara australis*. *Plant Signal. Behav*. **8**: e23279. doi: 10.4161/psb.23279.
113. Park S, Byeon Y, Lee HY, *et al.* (2014) Cloning and characterization of a serotonin *N*-acetyltransferase from a gymnosperm, loblolly pine (*Pinus taeda*). *J. Pineal Res*. **57**: 348-355. doi: 10.1111/jpi.12174.
114. Conti A, Tettamanti C, Singaravel M, *et al.* (2002) Melatonin: an ubiquitous and evolutionary hormone. In: *Treatise on Pineal Gland and Melatonin* (Haldar C, Singaravel M, Maitra SK, eds.), Science Publishers, Enfield – Plymouth, pp. 105 - 143.
115. Reiter RJ, Tan D-X (2002) Melatonin: an antioxidant in edible plants. *Ann. N.Y. Acad. Sci*. **957**: 341-344. doi: 10.1111/j.1749-6632.2002.tb02938.x.
116. Reiter RJ, Tan D-X, Burkhardt S, *et al.* (2001) Melatonin in plants. *Nutr. Rev*. **59**: 286-290. doi: 10.1111/j.1753-4887.2001.tb07018.x.
117. Caniato R, Filippini R, Piovan A, *et al.* (2003) Melatonin in plants. *Adv. Exp. Med. Biol*. **527**: 593-597.
118. Kolár J, Machácková I (2005) Melatonin in higher plants: occurrence and possible functions. *J. Pineal Res*. **39**: 333-341. doi: 10.1111/j.1600-079X.2005.00276.x.
119. Arnao MB, Hernández-Ruiz J (2006) The physiological function of melatonin in plants. *Plant Signal. Behav*. **1**: 89-95.
120. Reiter RJ, Tan D-X, Manchester LC, *et al.* (2007) Melatonin in edible plants (phytomelatonin): Identification, concentrations, bioavailability and proposed functions. *World Rev. Nutr. Diet*. **97**: 211-230. doi: 10.1159/000097917.
121. Paredes SD, Korkmaz A, Manchester LC, *et al.* (2009) Phytomelatonin: a review. *J. Exp. Bot*. **60**: 57-69. doi: 10.1093/jxb/ern284.
122. Tan D-X, Hardeland R, Manchester LC, *et al.* (2012) Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *J. Exp. Bot*. **63**: 577-97. doi: 10.1093/jxb/err256.
123. Zhang N, Sun Q, Zhang H, *et al.* (2015) Roles of melatonin in abiotic stress resistance in plants. *J. Exp. Bot*. **66**: 647-656. doi: 10.1093/jxb/eru336.
124. Reiter RJ, Tan D-X, Zhou Z, *et al.* (2015) Phytomelatonin: assisting plants to survive and thrive. *Molecules* **20**: 7396-7437. doi: 10.3390/molecules20047396.
125. Arnao MB, Hernández-Ruiz J (2015) Functions of melatonin in plants: a review. *J. Pineal Res*. **59**: 133-150. doi: 10.1111/jpi.12253.
126. Kaur H, Mukherjee S, Baluska F, *et al.* (2015) Regulatory roles of serotonin and melatonin in abiotic stress tolerance in plants. *Plant Signal. Behav*. **10**: e1049788. doi: 10.1080/15592324.2015.
127. Nawaz MA, Huang Y, Bie Z, *et al.* (2016) Melatonin: Current status and future perspectives in plant science. *Front. Plant Sci*. **6**: 1230. doi: 10.3389/fpls.2015.01230.
128. Hardeland R (2016) Melatonin – another phytohormone? *J. Bot. Sci*. **5**: 20-23.

129. Arnao MB, Hernández-Ruiz J (2018) Melatonin and its relationship to plant hormones. *Ann. Bot*. **121**: 195-207. doi: 10.1093/aob/mcx114.
130. Wang Y, Reiter RJ, Chan Z (2018) Phytomelatonin: a universal abiotic stress regulator. *J. Exp. Bot*. **69**: 963-974. doi: 10.1093/jxb/erx473.
131. Fan J, Xie Y, Zhang Z, *et al.* (2018) Melatonin: A multifunctional factor in plants. *Int. J. Mol. Sci*. **19**: E1528. doi: 10.3390/ijms19051528.
132. Yu Y, Lv Y, Shi Y, *et al.* (2018) The role of phyto-melatonin and related metabolites in response to stress. *Molecules* **23**: E1887. doi: 10.3390/molecules23081887.
133. Mukherjee S (2018) Novel perspectives on the molecular crosstalk mechanisms of serotonin and melatonin in plants. *Plant Physiol. Biochem*. **132**: 33-45. doi: 10.1016/j.plaphy.2018.08.031.
134. Sharif R, Xie C, Zhang H, *et al.* (2018) Melatonin and its effects on plant systems. *Molecules* **23**: E2352. doi: 10.3390/molecules23092352.
135. Kanwar MK, Yu J, Zhou J (2018) Phytomelatonin: Recent advances and future prospects. *J. Pineal Res*. **65**: e12526. doi: 10.1111/jpi.12526.
136. Arnao MB, Hernández-Ruiz J (2019) Melatonin: A new plant hormone and/or a plant master regulator? *Trends Plant Sci*. **24**: 38-48. doi: 10.1016/j.tplants.2018.10.010.
137. Zhan H, Nie X, Zhang T, *et al.* (2019) Melatonin: A small molecule but important for salt stress tolerance in plants. *Int. J. Mol. Sci*. **20**: E709. doi: 10.3390/ijms20030709.
138. Debnath B, Islam W, Li M, *et al.* (2019) Melatonin mediates enhancement of stress tolerance in plants. *Int. J. Mol. Sci*. **20**: E1040. doi: 10.3390/ijms20051040.
139. Li J, Liu J, Zhu T, *et al.* (2019) The role of melatonin in salt stress responses. *Int. J. Mol. Sci*. **20**: E1735. doi: 10.3390/ijms20071735.
140. Hardeland R (2005) Antioxidative protection by melatonin – Multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine* **27**: 119-130.
141. Hardeland R, Cardinali DP, Srinivasan V, *et al.* (2011) Melatonin – A pleiotropic, orchestrating regulator molecule. *Prog. Neurobiol*. **93**: 350-384. doi: 10.1016/j.pneurobio.2010.12.004.
142. Lee HY, Byeon Y, Back K (2014) Melatonin as a signal molecule triggering defense responses against pathogen attack in *Arabidopsis* and tobacco. *J. Pineal Res*. **57**: 262-268. doi: 10.1111/jpi.12165.
143. Lee HY, Byeon Y, Tan D-X, *et al.* (2015) *Arabidopsis* serotonin *N*-acetyltransferase knockout mutant plants exhibit decreased melatonin and salicylic acid levels resulting in susceptibility to an avirulent pathogen. *J. Pineal Res*. **58**: 291-299. doi: 10.1111/jpi.12231.
144. Qian Y, Tan D-X, Reiter RJ, *et al.* (2015) Comparative metabolomic analysis highlights the involvement of sugars and glycerol in melatonin-mediated innate immunity against bacterial pathogen in *Arabidopsis*. *Sci. Rep*. **5**: 15815. doi: 10.1038/srep15815.
145. Shi H, Chen Y, Tan D-X, *et al.* (2015) Melatonin induces nitric oxide and the potential mechanisms relate to innate immunity against bacterial pathogen infection in *Arabidopsis*. *J. Pineal Res*. **59**: 102-108. doi: 10.1111/jpi.12244.
146. Zhao H, Xu L, Su T, *et al.* (2015). Melatonin regulates carbohydrate metabolism and defenses against *Pseudomonas syringae* pv. tomato DC3000 infection in *Arabidopsis thaliana*. *J. Pineal Res*. **59**: 109-119. doi: 10.1111/jpi.12245.
147. Shi H, Qian Y, Tan D-X, *et al.* (2015) Melatonin induces the transcripts of CBF/DREB1s and their involvement in both abiotic and biotic stresses in *Arabidopsis*. *J. Pineal Res*. **59**: 334-342. doi: 10.1111/jpi.12262.

148. Shi H, Chen K, Wei Y, *et al.* (2016) Fundamental issues of melatonin-mediated stress signaling in plants. *Front. Plant Sci*. **7**: 1124. doi: 10.3389/fpls.2016.01124.
149. Wei Y, Hu W, Wang Q, *et al.* (2017) Identification, transcriptional and functional analysis of heat-shock protein 90s in banana (*Musa acuminata* L.) highlight their novel role in melatonin-mediated plant response to Fusarium wilt. *J. Pineal Res*. **62**: e12367. doi: 10.1111/jpi.12367.
150. Mandal MK, Suren H, Ward B, *et al.* (2018) Differential roles of melatonin in plant-host resistance and pathogen suppression in cucurbits. *J. Pineal Res*. **65**: e12505. doi: 10.1111/jpi.12505.
151. Chen X, Sun C, Laborda P, *et al.* (2018) Melatonin treatment inhibits the growth of *Xanthomonas oryzae* pv. *oryzae*. *Front. Microbiol*. **9**: 2280. doi: 10.3389/fmicb.2018.02280.
152. Ullah A, Manghwar H, Shaban M, *et al* (2018) Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environ. Sci. Pollut. Res. Int*. **25**: 33103-33118. doi: 10.1007/s11356-018-3364-5.
153. Ku YS, Sintaha M, Cheung MY, *et al.* (2018) Plant hormone signaling crosstalks between biotic and abiotic stress responses. *Int. J. Mol. Sci*. **19**: E3206. doi: 10.3390/ijms19103206.
154. Weeda S, Zhang N, Zhao X, *et al.* (2014). *Arabidopsis* transcriptome analysis reveals key roles of melatonin in plant defense systems. *PLoS One* **9**: e93462. doi: 10.1371/journal.pone.0093462.
155. Erland LAE, Shukla MR, Singh AS, *et al.* (2018) Melatonin and serotonin: Mediators in the symphony of plant morphogenesis. *J. Pineal Res*. **64**: e12452. doi: 10.1111/jpi.12452.
156. Tan D-X, Hardeland R, Back K, *et al.* (2016) On the significance of an alternate pathway of melatonin synthesis via 5-methoxytryptamine: comparisons across species. *J. Pineal Res*. **61**: 27-40. doi: 10.1111/jpi.12336.
157. Okazaki M, Higuchi K, Aouini A, *et al.* (2010) Lowering intercellular melatonin levels by transgenic analysis of indoleamine 2,3-dioxygenase from rice in tomato plants. *J. Pineal Res*. **49**: 239-247. doi: 10.1111/j.1600-079X.2010.00788.x.
158. Tan D-X, Manchester LC, Di Mascio P, *et al.* (2007) Novel rhythms of N^1-acetyl-N^2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation. *FASEB J*. **21**: 1724-1729. doi: 10.1096/fj.06-7745com.
159. Byeon Y, Back K (2015) Molecular cloning of melatonin 2-hydroxylase responsible for 2-hydroxymelatonin production in rice (*Oryza sativa*). *J. Pineal Res*. **58**: 343-351. doi: 10.1111/jpi.12220.
160. Byeon Y, Lee HY, Hwang OJ, *et al* (2015) Coordinated regulation of melatonin synthesis and degradation genes in rice leaves in response to cadmium treatment. *J. Pineal Res*. **58**: 470-478. doi: 10.1111/jpi.12232.
161. Byeon Y, Tan D-X, Reiter RJ, *et al.* (2015) Predominance of 2-hydroxymelatonin over melatonin in plants. *J. Pineal Res*. **59**: 448-454. doi: 10.1111/jpi.12274.
162. Yang Y, Zhou R, Park SY, *et al* (2017) 2-Hydroxymelatonin, a predominant hydroxylated melatonin metabolite in plants, shows antitumor activity against human colorectal cancer cells. *Molecules* **22**: E453. doi: 10.3390/molecules22030453.
163. Slominski AT, Semak I, Fischer TW, *et al.* (2017) Metabolism of melatonin in the skin: Why is it important? *Exp. Dermatol*. **26**: 563-568. doi: 10.1111/exd.13208.
164. Horstman JA, Wrona MZ, Dryhurst G (2002) Further insights into the reaction of melatonin with hydroxyl radical. *Bioorg. Chem*. **30**: 371-382. doi: 10.1016/S0045-2068(02)00511-4.

165. Hardeland R (2008) Melatonin, hormone of darkness and more: occurrence, control mechanisms, actions and bioactive metabolites. *Cell. Mol. Life Sci*. **65**: 2001-2018. doi: 10.1007/s00018-008-8001-x.
166. Pérez-González A, Galano A, Alvarez-Idaboy JR, *et al.* (2017) Radical-trapping and preventive antioxidant effects of 2-hydroxymelatonin and 4-hydroxymelatonin: Contributions to the melatonin protection against oxidative stress. *Biochim. Biophys. Acta* **1861**: 2206-2217. doi: 10.1016/j.bbagen.2017.06.016.
167. Lee HJ, Back K (2016) 2-Hydroxymelatonin promotes the resistance of rice plant to multiple simultaneous abiotic stresses (combined cold and drought). *J. Pineal Res*. **61**: 303-316. doi: 10.1111/jpi.12347.
168. Lee K, Zawadzka A, Czarnocki Z, *et al.* (2016) Molecular cloning of melatonin 3-hydroxylase and its production of cyclic 3-hydroxymelatonin in rice (*Oryza sativa*). *J. Pineal Res*. **61**: 470-478. doi: 10.1111/jpi.12361.
169. Wei J, Li D-X, Zhang J-R, *et al.* (2018) Phytomelatonin receptor PMTR1-mediated signaling regulates stomatal closure in *Arabidopsis thaliana*. *J. Pineal Res*. **65**: e12500. doi: 10.1111/jpi.12500.

Please cite this paper as:
Hardeland, R. 2019. Melatonin in the evolution of plants and other phototrophs. Melatonin Research. 2, 3 (Aug. 2019), 10-36. DOI:https://doi.org/https://doi.org/10.32794/mr11250029.