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REVIEW ARTICLE

Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes

Abstract: Mitochondria and chloroplasts are major sources of free radical generation in living organisms. Because of this, these organelles require strong protection from free radicals and associated oxidative stress. Melatonin is a potent free radical scavenger and antioxidant. It meets the criteria as a mitochondrial and chloroplast antioxidant. Evidence has emerged to show that both mitochondria and chloroplasts may have the capacity to synthesize and metabolize melatonin. The activity of arylalkylamine *N*-acetyltransferase (AANAT), the reported rate-limiting enzyme in melatonin synthesis, has been identified in mitochondria, and high levels of melatonin have also been found in this organelle. From an evolutionary point of view, the precursor of mitochondria probably is the purple nonsulfur bacterium, particularly, *Rhodospirillum rubrum,* and chloroplasts are probably the descendents of cyanobacteria. These bacterial species were endosymbionts of host proto-eukaryotes and gradually transformed into cellular organelles, that is, mitochondria and chloroplasts, respectively, thereby giving rise to eukaryotic cells. Of special importance, both purple nonsulfur bacteria (*R. rubrum*) and cyanobacteria synthesize melatonin. The enzyme activities required for melatonin synthesis have also been detected in these primitive species. It is our hypothesis that mitochondria and chloroplasts are the original sites of melatonin synthesis in the early stage of endosymbiotic organisms; this synthetic capacity was carried into host eukaryotes by the above-mentioned bacteria. Moreover, their melatonin biosynthetic capacities have been preserved during evolution. In most, if not in all cells, mitochondria and chloroplasts may continue to be the primary sites of melatonin generation. Melatonin production in other cellular compartments may have derived from mitochondria and chloroplasts. On the basis of this hypothesis, it is also possible to explain why plants typically have higher melatonin levels than do animals. In plants, both chloroplasts and mitochondria likely synthesize melatonin, while animal cells contain only mitochondria. The high levels of melatonin produced by mitochondria and chloroplasts are used to protect these important cellular organelles against oxidative stress and preserve their physiological functions. The superior beneficial effects of melatonin in both mitochondria and chloroplasts have been frequently reported.

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Melatonin, a unique antioxidant

Since the discovery of melatonin as an unusually effective free radical scavenger and a natural antioxidant in 1993 [1], thousands of publications have confirmed this important observation, and the concept that melatonin provides essential protection against oxidative stress has been widely accepted by scientists and investigators [2–11]. Free radical generation and scavenging are among the most basic activities occurring in cells and cellular organelles. As a result, the unique antioxidant properties of melatonin make it one of the basic substances in all living organisms from bacteria to humans [12, 13]. Melatonin is a highly conserved molecule, and its origin can be traced back an estimated 2.5 billion years [14]. It is speculated that melatonin evolved at the time organisms began their transition from an anaerobic to aerobic metabolism. The original and primary function of melatonin in organisms is to serve as an antioxidant to detoxify the free radicals generated during the process of aerobic metabolism with the other functions of melatonin [15–17] presumably being acquired during evolution [14]. As evidence of its early origin,

melatonin has been found in primitive bacteria, unicellular organisms, and green algae [18]. The function of melatonin in these organisms is proven to serve as an antioxidant [7]. In addition to its presence in these primitive organisms, melatonin is also found in plants, fungi, insects, nematodes, and vertebrates including mammals [18–20]. Both endogenously produced and exogenously administered melatonin effectively protects against oxidative stress in each of these species.

Compared to the classic antioxidants such as vitamin C, vitamin E, and glutathione, melatonin exhibits a more potent antioxidant capacity to reduce oxidative injury, especially under in vivo conditions [21–28]. The superior antioxidant capacity of melatonin is, at least partially, attributed to what is referred to as the cascade reaction when scavenging free radicals [29] (Fig. 1). This specific cascade of scavenging reactions of melatonin is a result of its unique metabolism. Melatonin interacts with variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS) including the hydroxyl radical ($HO^{\cdot}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), peroxynitrite anion ($ONOO^-$), and nitric oxide (NO). The resulting products of these reactions are cyclic 3-hydroxymelatonin (C-3HOM) and other hydroxylated melatonin metabolites, N^1-acetyl-N^2-formyl-5-methoxyknuramine (AFMK), and *N*-acetyl-5-methoxy-knuramine (AMK; Fig. 1). These metabolites, like their precursor melatonin, also function as radical scavengers. Some of them including C-3HOM and AMK are more aggressive than melatonin regarding their capacity to scavenge oxidants [30, 31]. It is estimated that via the cascade reaction, one melatonin molecule may scavenge up to 10 free radicals [18], which contrasts with the classic antioxidants because they typically detoxify one radical per molecule.

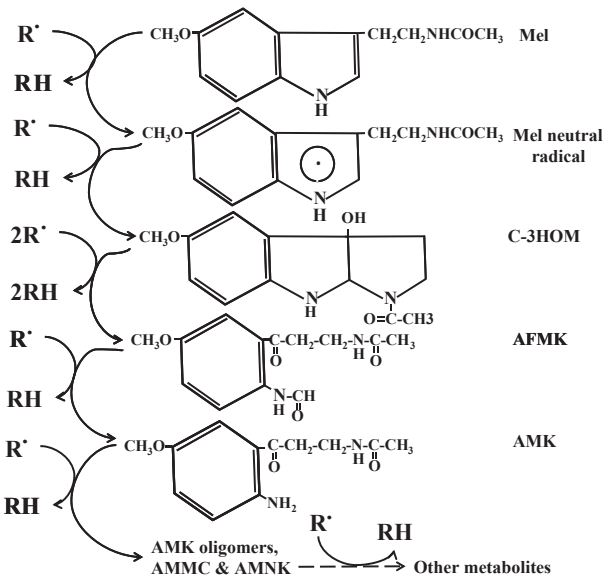

Fig. 1. Cascade reaction of melatonin interaction with free radicals and its metabolites. R·, radical; RH, reduced agent; Mel, melatonin; C-3HOM, cyclic 3-hydroxymelatonin; AFMK, N^1-acetyl-N^2-formyl-5methoxyknuramine; AMK, *N*-acetyl-5-methoxyknuramine; AMMC, 3-acetamidomethyl-6-methoxycinnolinone; AMNK, N^1-acetyl-5-methoxy-3-nitrokynuramine; dash arrow, unidentified reactions.

Other aspects of melatonin which explain its ubiquitous actions as a potent antioxidant are its subcellular distribution plus its unique ability to recycle other classic antioxidants. The conventional antioxidants, that is, vitamin C, vitamin E, coenzyme Q10 (Q_{10}), and glutathione, have limited intracellular distributions. For example, vitamin C is water-soluble and mainly locates in the cytosol. Vitamin E and Q10 are lipophilic and are mainly distributed in cell membranes. Their unique distributions limit them as all-round antioxidants. For example, if the oxidative reaction occurs in the cytosol and water-soluble antioxidants are exhausted, and even though an abundance of lipophilic antioxidants may exist in the cellular membranes, they are not helpful in participating in cytosolic antioxidative defense processes. The limited distribution of antioxidants in specific subcellular compartments in a sense is wasteful of the beneficial action of an antioxidant. If a molecule could link the function of antioxidants, that is, network, in different subcellular environments, it could dramatically amplify the antioxidative capacity of cells as a whole. Melatonin seems to be one such natural molecule. Melatonin is amphiphilic and distributes both in the aqueous cytosol and in lipid-rich membranes [32–34]. Evidence has accumulated suggesting that melatonin may recycle several oxidized antioxidants including vitamin C, vitamin E, glutathione, and NADH, and these antioxidants reportedly also recycle the melatonin neutral radical [24, 35–42].

It seems that this bidirectional recycling by melatonin may depend on conditions, and it exhibits a concentration equilibrium. This suggests that when antioxidants in the cytosol are exhausted by excessive cytosolic oxidative stress, the lipophilic antioxidants resident in other cellular compartments could provide antioxidative protection in the cytosol using melatonin as the bridge to recycle water-soluble antioxidants and vice versa. This would be classified as antioxidant network (Fig. 2). The synergistic effects of melatonin with other antioxidants have been reported under several experimental conditions [43–47].

Melatonin has been successfully used to protect against oxidative injuries in a very wide variety of oxidative models in animals including ischemia/reperfusion damage in the important organs, for example, heart, lung, liver, muscle, and brain [48–56], diabetes [57–61], neurodegenerative diseases [62–66], and organ damage caused by safrole [67, 68] and other toxic chemicals [69–72]. In addition, clinical trials in humans have reported that melatonin effectively reduces the severity of diseases and disorders in which their etiologies are associated with free radical damage or oxidative stress [73–77]. While the results of these experimental and clinical studies clearly document the ability of melatonin to reduce oxidative damage resulting from a variety of oxidative insults in widely different organs, the possibility exists that the protective actions of the indole are related exclusively to its radical scavenging activities as well as to its ability to work in conjunction with other antioxidants via the network described above.

Recently, melatonin's presence in and association with plant physiology has been a rapidly developing field of research [78]. Plants contain high levels of melatonin.

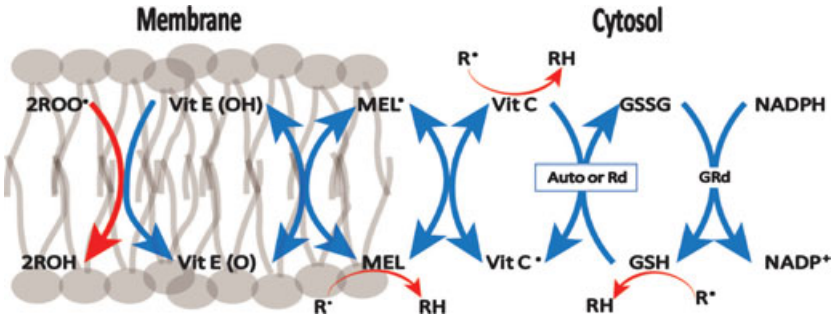

Fig. 2. Proposed cellular antioxidant network. Melatonin is an amphiphilic molecule. The cellular hydrophilic antioxidants may be functionally connected with cellular lipophilic antioxidants within cells via melatonin as a bridge. MEL, melatonin; MEL˙, melatonin neutral radical; Vit E (O) oxidized vitamin E; Vit E (OH), reduced vitamin E; Vit C (O), oxidized vitamin C; Vit C (OH), oxidized vitamin C; ROO˙, Proxy radical; R˙, radical; RH, reduced agent; Auto or Rd, automatically or via reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GRd, glutathione reductase.

A major portion of the melatonin in plants is synthesized by the plant itself; this has been proven in rice and in other plants. All enzymes required for melatonin biosynthesis have been identified in plants. Another portion of melatonin in plants may be a result of its absorption through the roots. Microorganisms in soil synthesize melatonin thereby providing a source for plants. The decomposed microorganisms and vegetation inevitably release melatonin into soil. It is well documented that plants extract melatonin from their surroundings, for example, from water [79].

Melatonin in plants, as in animals, has a variety of important functions; however, the primary function of melatonin in plants is to serve as a first-line defense against oxidative stress. Mitochondria and chloroplasts are the primary source of free radical production and oxidative stress in living organisms. The free radicals are continuously generated during cellular respiration in mitochondria and during photosynthesis in chloroplasts. To protect against oxidative injuries and preserve the functions of these organelles, the noxious free radicals must be detoxified via antioxidant processes. Melatonin functions as a major defense against free radicals in plants as in animals; we predict it has a pivotal role in defending against radicals at both the mitochondrial and chloroplast levels. The protective effects of melatonin on mitochondrial oxidative injury are already well established [80–84], and its beneficial effects against chlorophyll oxidation [5, 41, 85–87] have also been recently reported. Considering the evolutionary origin of mitochondria and chloroplasts from bacteria (which produce melatonin), it seems that these intracellular organelles may well have the capacity to synthesize the indoleamine in all cells. Herein, we summarize the evidence that supports melatonin production by mitochondria and chloroplasts.

Roles of melatonin in mitochondria and chloroplasts

The primary function of mitochondria is to generate bioenergy, ATP, with high efficiency. This process is referred to as oxidative phosphorylation coupling. During this process, energy contained in the high-voltage electrons is released stepwise via an electron transport chain (ETC) and is used to pump protons across the inner mitochondrial membrane against a gradient. The energy built-up as a result of the proton gradient is then used to produce ATP. At the end of the ETC, the electrons are transferred to oxygen, and finally, oxygen is reduced to water. However, the ETC is not flawless, and some electrons invariably leak from the ETC to partially reduce oxygen- or nitrogen-related substances forming ROS and RNS, respectively [88]. The excessive production and accumulation of ROS and RNS in mitochondria eventually alter their function. If the ROS and RNS are not neutralized in a timely manner, the damage jeopardizes the functions of mitochondria and finally destroys these organelles [89].

During evolution, mitochondria developed several mechanisms to protect against ROS generation and oxidative injury. Melatonin may be a critical molecule that preserves mitochondrial integrity and physiology [90]. The roles that melatonin has at the mitochondrial level include (i) protection against mitochondrial oxidative stress and apoptosis via scavenging ROS and RNS; (ii) increasing the efficiency of ATP production by accelerating electron flow through the ETC by a mechanism that promotes the activities of complexes I, III, and V [91–93]; (iii) maintaining an optimal membrane potential across the inner mitochondrial membrane by regulating the mitochondrial permeability transition pore (MPTP) [94–96]. Melatonin actually exhibits dual effects on the MPTP. Under normal conditions, melatonin activates MPTP and mildly reduces the mitochondrial membrane potential [97, 98]. Under oxidative stress conditions that damage MPTP function, melatonin significantly inhibits the MPTP and thus preserves the membrane potential to avoid mitochondrial collapse [95, 99–103]. The dual functions of melatonin on MPTP establish the indoleamine as an excellent molecule, which balances mitochondrial membrane potential and maintains the optimal function of mitochondria to generate ATP under both favorable and unfavorable conditions; (iv) in brown adipose tissue (BAT) of mammals, melatonin promotes oxidative phosphorylation uncoupling by promoting the activity of uncoupling protein 1 (UCP1) [104]. This process converts energy stored in fat into heat. This is important, especially for newborns and hibernators, allow-

ing them to tolerate cold temperatures. In addition, the uncoupling effect of melatonin in BAT mitochondria may have a significant impact on obesity. It is speculated that increased weight gain may relate to reduced melatonin production, which in turn limits the amount of BAT; because BAT burns excess calories, its reduction causes white fat accumulation [105, 106].

Because melatonin clearly has several important functions in mitochondria, it can be deduced that a fundamental purpose of melatonin in mitochondria is to preserve the physiological functions of this organelle under both normal and stressful conditions. As an example, melatonin administration to aged animals restores their hepatic and neuronal mitochondrial physiology to youthful levels including increasing the active mitochondrial population as well as their functions [107–109]. The beneficial actions of melatonin are also apparent in transgenic mouse model of Alzheimer's disease (a mouse with Swedish mutant amyloid precursor protein). This animal model exhibits extensive mitochondrial dysfunction. The malfunctioning mitochondria are associated with cognitive decline and the Alzheimer's pathologies these mice display. It was recently observed that melatonin administration to these mice nearly completely restores mitochondrial function in terms of respiratory rate, membrane potential, ROS production, and ATP values to normal levels [110].

As to the roles of melatonin in chloroplasts, these have not been adequately examined because research on plant melatonin is still in its early stages. However, several studies have uncovered evidence that melatonin effectively prevents chlorophyll deterioration and preserves chloroplast physiology. The destruction of chlorophyll involves oxidative stress. By preserving chloroplast integrity, melatonin promotes photosynthesis, which is essential for the survival of plants [78, 85].

Protective mechanisms of melatonin in mitochondria and chloroplasts

Major sources of ROS and RNS derive from the mitochondria or chloroplasts, and these toxic reactants are by-products of energy metabolism. These toxic by-products, if not neutralized, morphologically and functionally damage mitochondria and chloroplasts. In addition, when these oxidants, especially the long-lived H_2O_2, diffuse out of these organelles, they also injure molecules in other subcellular compartments. One of the protective mechanisms of melatonin in mitochondria and chloroplasts is its direct interaction with free radicals. Most of these radicals, especially $HO^{\cdot}$, have extremely short half-lives in the range of a fraction of a nsec. Thus, they rapidly destroy any molecule they encounter within a distance of several Angstroms (Å). Thus, to effectively scavenge $HO^{\cdot}$, melatonin must have two essential characteristics. First melatonin must be present in mitochondria to be in the immediate vicinity of where radicals are generated, so it can provide on-site protection. Second, melatonin must rapidly react with $HO^{\cdot}$. Melatonin obviously satisfies both criteria. High levels of melatonin have been identified in mitochondria [111, 112] where it clearly quenches free radicals. The rate constant of melatonin's reaction with $HO^{\cdot}$ is calculated to be 1.3 -4×10^{10}/M/s, that is, melatonin scavengers $HO^{\cdot}$ at a diffusion controlled rate [36, 113, 114].

In addition to a direct interaction with ROS and RNS, several other protective mechanisms of melatonin in mitochondria and chloroplasts have been reported. Melatonin down-regulates free radical production via two separate pathways, a process referred to as free radical avoidance. One of these pathways is that melatonin accelerates electron flow through the ETC. Accelerating electron flow in the ETC reduces the electron leakage from the chain and avoids partially reducing oxygen and, thus, limiting the generation of ROS and RNS. To achieve this, melatonin promotes the activities of complex I and complex III [93, 115–117], which are the main electron leakage sites in the ETC. For example, complex I specific inhibitor, rotenone, and complex III specific inhibitor, antimycin A, jeopardize electron flow in the ETC and result in large quantities of radicals being formed [118–120]. Melatonin supplementation to cells or organisms effectively preserves mitochondrial function and reduces oxidative injury induced by these electron flow inhibitors [121–123]. The second pathway is that melatonin mildly lowers the mitochondrial membrane potential. A high mitochondrial membrane potential also slows down the electron flow and results in electron leakage to generate free radicals; thus, a moderate down-regulation of the mitochondrial membrane potential significantly reduces free radical formation [89]. Recall, melatonin lowers mitochondrial membrane potential under normal conditions. This process is associated with mitochondrial oxidative phosphorylation uncoupling.

The uncoupling effects of melatonin in BAT were observed decades ago. Recently, this effect of melatonin has been confirmed in other cells. The exact mechanisms by which melatonin uncouples oxidative phosphorylation are not fully understood. It may be related to the activation of uncoupling protein $(UCP)_2$ and UCP_3. Activation of UCP_2 or UCP_3 causes a moderate uncoupling and significantly lowers radical formation [124, 125]. Melatonin promotes the activity of UCP_2 and UCP_3 (D. Acuna-Castroviejo and G. Escames, unpublished data). Thus, melatonin has the capacity to down-regulating radical formation in mitochondria [126]. As a mitochondrial antioxidant, a characteristic of radical avoidance is more efficiency than only scavenging radicals. By promoting radical avoidance while also effectively functioning as a scavenger, melatonin has greater efficiency in limiting oxidative damage than do other antioxidants in mitochondria [111, 127].

Up-regulation of antioxidant enzymes and down-regulation of prooxidant enzymes are other mechanisms that contribute to the protective effects of melatonin on mitochondria. Melatonin enhances the activities of several antioxidant enzymes that are involved in mitochondrial metabolism including superoxide dismutase [107, 128, 129], catalase [130], glutathione peroxidase and heme oxygenase1 [131]. Conversely, melatonin inhibits activities of some prooxidant enzymes including inducible nitric oxide synthase [132, 133], cyclo-oxidase 2 (COX_2), and myeloperoxidase [134–137]. The effects of melatonin on these enzymes likely contribute, at least partially, to its protective effects against mitochondrial oxidative stress. Finally,

melatonin may work directly on a melatonin receptor in the mitochondria. Using a mouse genetic model of Huntington's disease, Wang et al. [62] identified the MT_1 receptor in mitochondria. The protective effects of melatonin on mutant Huntington-mediated toxicity were shown to be partially mediated by the mitochondrial MT_1. Thus, blockage of the mitochondrial MT_1 using luzindole dampened the protective effects of melatonin. The proposed mechanisms involve melatonin's ability to reduce the loss of mitochondrial membrane potential and functions induced by the mutant Huntington gene and its action in reducing the release of mitochondrial proapoptotic factors.

Evidence for the biosynthesis and metabolism of melatonin in mitochondria and chloroplasts

A potential association between melatonin biosynthesis and mitochondria in pinealocytes was investigated decades ago. Several studies have reported that melatonin production strongly correlated with alterations in mitochondrial ultrastructure. Enlarged mitochondria always associate with functional pinealocytes which produce more melatonin [138–140]. In all species investigated, melatonin production in the pineal gland is at its peak during the night and the volume of pineal mitochondria is also greater at this time than that they are during the photophase [141]. When the superior cervical ganglia are surgically removed the pineal gland loses its ability to synthesize melatonin, and the relative volumes of mitochondria correspondently shrink [142]. Similarly, in cultured rat pineal gland, the addition of vasoactive intestinal peptide to the culture medium elevated the conversion of serotonin to melatonin and its subsequent secretion. Again, as in the in vivo studies, the volumes of the mitochondria in cultured pineal glands were also amplified [143]. At the time these observations were made, the alteration in mitochondrial ultrastructure with the changes in melatonin production in pinealocytes was interpreted to mean that the cells required more energy for melatonin synthesis. However, it is well documented that the volume changes of the mitochondria are not positively correlated with their metabolic states [144]. For example, at state 3, the metabolic activity of mitochondria is higher than that at state 4; however, the mitochondrial size in state 3 is smaller than in state 4. Therefore, the enlarged mitochondria during night or at the peak of melatonin production have little to do with energy metabolism. It appears that the alterations of mitochondrial ultrastructure are to meet the requirements of melatonin production in cells.

A close relationship between melatonin and mitochondria was also uncovered in several studies. Melatonin can directly bind to the mitochondria of the pigeon brain. A comparison of melatonin in several subcellular organelles showed that majority of melatonin binding was located in the mitochondrial fraction [145]. The binding of melatonin to mitochondria was also reported to occur in the spleen of the guinea pig [146]. Its occurrence in a bird (pigeon) and in a mammal (guinea pig) and in different organs suggests that the association may be a common finding if systematically investigated. Recently, MT_1 melatonin receptor was found in mitochondria of mice [62]. While these results do not prove the synthesis of melatonin in mitochondria, they do document the association of the indoleamine with this critical organelle.

The data from Martin et al. [111] possibly raise a fundamental question as to whether mitochondria have the ability to generate melatonin. They reported that when mice were given melatonin, the level of the indoleamine in mitochondria significantly increased over levels in the serum. It was speculated that the melatonin in mitochondria was exclusively extracted from the blood. However, an alternative interpretation also is not precluded, that is, the extremely high levels of melatonin in mitochondria are an additive result of mitochondria-generated melatonin plus serum-extracted melatonin.

For mitochondria to produce melatonin, the machinery for the synthesis of the indoleamine would require a series of essential enzymes. The rate-limiting enzyme of melatonin synthesis, arylalkylamine *N*-acetyltransferase (AANAT), has been at least tentatively identified in mitochondria [147]. With the aid of electron microscopy and enzyme histochemistry, an intense immunoreactivity for AANAT was found to be exclusively located in the outer and inner membranes and intermembrane space of pinealocyte mitochondria (Fig. 3). AANAT is generally considered to be the rate-limiting enzyme in melatonin synthesis and appears to be located at the site where cellular respiration occurs. The observation was confirmed in a follow-up investigation by the same group [148]. This indicates, if the above observations are reliable, that mitochondria per se may at least produce the immediate precursor of

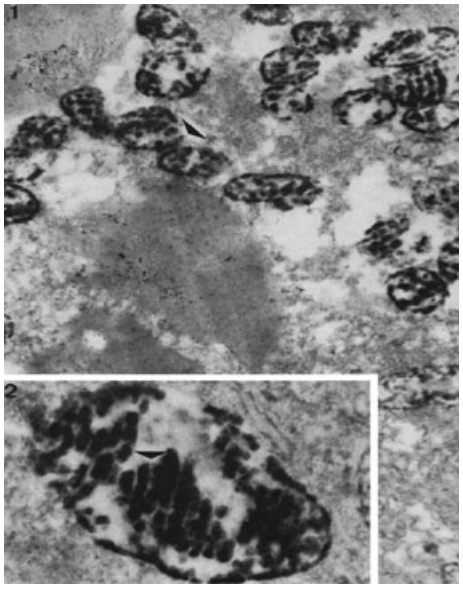

Fig. 3. Electron microscopic evidence of immunocytochemically identified arylalkylamine *N*-acetyltransferase (AANAT) localization in pinealocytes. The AANAT reaction product was exclusively found in mitochondria. The low magnification figure illustrates the wide spread reaction product in all mitochondria (6500×). Insert provides additional detail (48,000×); The AANAT reaction product is situated mainly between the outer and inner membrane of a mitochondrion. From Kerenyi et al. [147].

melatonin, *N*-acetylserotonin. Interestingly, in the neonatal rat pineal gland, the first evidence of melatonin synthetic rhythm occurs concurrent with the initial appearance of the structurally identified mitochondria, that is, late in the suckling period [149, 150].

More direct evidence that mitochondria may be a site of melatonin production has emerged [112]. Using advanced technologies, extremely high daytime levels of melatonin were identified in the mitochondria of rat heart (Fig. 4). These animals had not been previously exposed to the exogenous melatonin, and therefore, it is likely that this high level of mitochondrial melatonin could not simply be explained by its extraction from daytime blood in which melatonin levels are generally low. With the aid of HPLC plus fluorescent detection, high levels of melatonin have been identified in rat liver and brain mitochondria by others [34]. These levels of melatonin are independent of the circulating melatonin of pineal origin. In addition to melatonin, its metabolite, AFMK, was also identified in the mitochondria. The level of mitochondrial AFMK appeared to be somewhat higher than that of melatonin (Fig. 4). The metabolic pathway by which melatonin is converted into AFMK in mitochondria is different from that in cells. For example, in neurons, this conversion is either via direct interaction of melatonin with free radicals or via a metabolism by enzymes, mainly, indolamine 2,3-dioxygenase (IDO) [151]. In mitochondria, this conversion is primarily via a pseudo-enzymatic process, that is, cytochrome *C* is the substance responsible for conversion of melatonin into AFMK. Cytochrome *C* is not a typical conventional enzyme, and how it metabolizes melatonin to AFMK is not fully understood. Considering the highly conserved nature of both cytochrome *C* and melatonin, the former may serve as the initial mechanism to breakdown melatonin in primitive organisms. Other enzymes involved in melatonin metabolism may have been acquired during evolution.

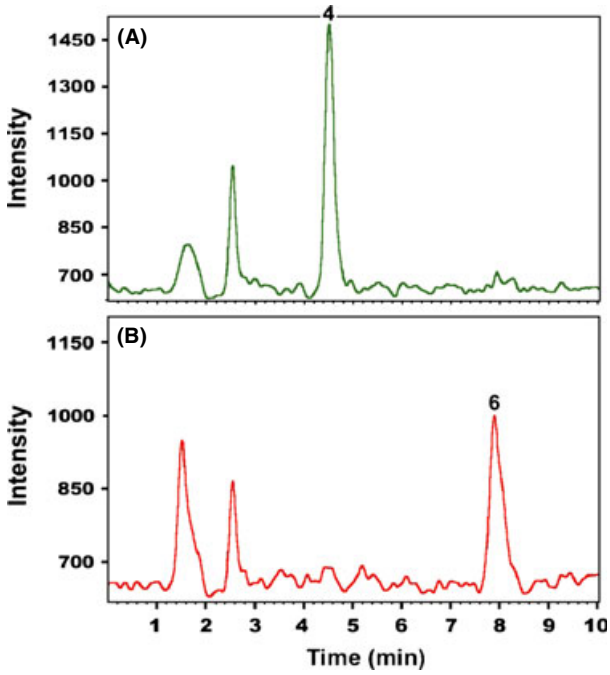

Fig. 4. LC-MS analysis of endogenous melatonin and its metabolites in heart mitochondria of male Wistar rat. Mass spectrometric analysis was carried out in SIM mode. Green – ion at m/z = 265 (A); red – ion at m/z = 233 (B). The peaks designated as 4 and 6 correspond to AFMK and melatonin, respectively. Semak et al. [112].

Recently, cytochrome P450 was also uncovered in mitochondria. This enzyme is partially responsible for the metabolism of melatonin to AFMK, 2-hydroxymelatonin, 6-hydroxymelatonin and other minor metabolites [152]. The evidence summarized here generally suggests that mitochondria as an organelle is similar to an intact cell in its capacity to synthesize, utilize, and metabolize melatonin. This may apply also to chloroplasts because they have a similar origin as mitochondria.

Evolutionary aspects of melatonin biosynthesis in mitochondria and chloroplasts

Different from other organelles in eukaryotes, mitochondria and chloroplasts have their own DNA, which is circular and similar to the DNA of bacteria. In addition, their ribosomes and tRNA molecules also share the similarity with bacteria. On the basis of these observations, the extracellular origins of mitochondria and chloroplasts were proposed by L Sagan (nee, L Margulis) in 1967 [153]. She suggested that both mitochondria and chloroplasts originated from bacteria via an endosymbiotic process with their ancestral eukaryotic host. The hypothesis of the endosymbiotic genesis of mitochondria and chloroplasts currently is accepted by mainstream scientists. It is believed that mitochondria are probably descendant from purple nonsulfur bacteria, particularly *Rhodospirillum rubrum,* and chloroplasts likely originated from cyanobacteria. A genomic phylogenetic analysis of *R. rubrum* indicates its close relationship with mitochondria, that is, closer than any alphaproteobacteria investigated [154]. Interestingly, *R. rubrum* is the first photosynthetic alphaproteobacterium shown to synthesize melatonin, and it does so with an obvious circadian rhythm [155]. In addition to *R. rubrum,* another purple nonsulfur bacterium species, *Erythrobacter longus*, also produces melatonin in a cyclic manner with peak values during darkness [156]. *Erythrobacter longus* is classified as an alphaproteobacterium, and cytochrome *C* oxidase has been found in this species. The spectral properties of its cytochrome *C* oxidase closely resemble those of mitochondria [157].

If the endosymbiotic genesis of mitochondria is valid, there is no reason to doubt that mitochondria per se may also synthesize melatonin. Evidence shows that melatonin's synthetic enzymes in some organisms are horizontally transferred during the endosymbiosis of bacteria. For example, in some organisms including fungi and unicellular algae, the gene homologs of AANAT are horizontally transferred from gram-positive bacteria [78]. We theorize that the melatonin synthetic ability of these organisms was originally acquired from other endosymbiotic species, which were finally transformed into cellular organelles including mitochondria and chloroplasts.

The melatonin synthetic pathway in plants is different from that in animals, as are its enzymes. AANAT of plants has been recently identified in rice plant [158]. Rice AANAT in many respects differs from the animal AANAT including the DNA sequence and enzymatic kinetics. It shares no sequence homology to any known AANAT genes in animals. Its origin is likely different from that in animals. However, rice plant AANAT gene shares 56% homology with that of cyanobacteria. Rice plant AANAT polypeptides present a positive transit sequence, which targets protein into chloroplasts. This indicates that some translocation mechanisms were developed to transport AANAT into chloroplasts. Similar to the proposed origin of mitochondria, chloroplasts are also believed to be the descendants of cyanobacteria, which originally endosymbiosed with the eukaryotic host cells such as green algae. Cyanobacteria are also melatonin-generating bacteria. Awantang and Tilden [159] have reported that this bacterium can synthesize melatonin with an obvious circadian rhythm in which melatonin concentration is high during the scotophase and low during the photophase. The melatonin synthetic activity of cyanobacteria has been confirmed in other reports [160, 161]. Furthermore, the enzyme activities for melatonin synthesis have also been found in these cyanobacteria. The evolutionary evidence strongly supports that as the descendant of cyanobacteria, chloroplasts may well have the capacity to produce melatonin.

It is our hypothesis that in most organisms, if not all, melatonin biosynthetic ability was originally transferred from mitochondria and chloroplasts to other compartments of the cell, for example, the cytosol. In the early stage of biosymbiosis, mitochondria and chloroplasts were the only sites of melatonin synthesis in cells. During evolution, the genes for melatonin biosynthesis contained in the *R. rubrum* or other purple nonsulfur photosynthetic bacteria, now called mitochondria, and cyanobacteria, now called chloroplasts, gradually were transferred (integrated) into the nuclear genome. In this manner, other cellular compartments acquired melatonin biosynthetic capability. It is well documented that, not only pinealocytes, but perhaps all cells and tissues produce melatonin [18], and this may be associated with the presence of mitochondria and chloroplasts. It is obvious that the melatonin synthetic genes were likely transferred into the nucleus from the mitochondrial genome or lost during evolution. Gene loss or transfer to the nucleus from mitochondria during evolution has been a common phenomenon [162]. However, the melatonin synthetic enzymes coded by nuclear DNA (originally transferred from the endosymbiotic bacteria) somehow were transported back to mitochondria, so they could produce melatonin. For example, AANAT activity has been identified in mitochondria as mentioned previously.

Mitochondria as the site of oxidative phosphorylation, ATP production and chloroplasts as sites of photosynthesis are the major sources of free radical generation in cells. They require strong protection against oxidative injury to preserve their physiological functions. It is logical that these organelles preserved their capacities to produce a highly effective antioxidant, that is, melatonin, during evolution. In some cells, mitochondria (or chloroplasts) may be the primary site for melatonin synthesis. Melatonin in other cellular compartments or in serum may be derived from mitochondria. This is hinted at by the observations of Kerenyi et al. [149, 150] where AANAT activity was found exclusively located in the mitochondria of pinealocytes (Fig. 3). If, as we hypothesize, that both mitochondria and chloroplasts are sites of melatonin synthesis, a plausible explanation can be given to a frequently asked question, that is, why are the melatonin levels so much higher in green plants than in animals? The answer may be that green plants have two organelles (mitochondria and chloroplasts) that produce melatonin, while animals have only mitochondria to perform this function.

Concluding remarks

Mitochondria as the powerhouses and chloroplasts as the photosynthetic sites are the major sources of free radical generation. They require strong protection from oxidizing molecules. Melatonin may be considered as a mitochondrial antioxidant because it not only directly scavenges and indirectly neutralizes free radicals, but it also reduces radical generation in mitochondria, via a phenomenon known as radical avoidance. To achieve radical avoidance, melatonin accelerates the electron flow through the ETC and slightly activates the MPTP. This may explain why melatonin is more protective of mitochondria against oxidative stress than other antioxidants. Studies have documented that melatonin restores the mitochondrial functions in aged animals and in animals with different pathological conditions. In addition to mitochondria, protective effects of melatonin on chloroplasts have also been revealed in a number of plants. Recent evidence implies that both mitochondria and chloroplasts may have the ability to synthesize melatonin. It is our hypothesis that mitochondria and chloroplasts were the initial sites of melatonin biosynthesis at the early stage of endosymbiosis. We also presume that the melatonin biosynthetic capacity has been preserved in these organelles during evolution. These cellular organelles likely require melatonin to provide on-site protection against oxidative stress and to preserve their important physiological functions. The ideas proposed herein deserve further investigation. The proposed production of melatonin by mitochondria and chloroplasts does not necessarily exclude the possibility that other areas of the cell may also be capable of melatonin generation.

References

1. Tan DX, Chen LD, Poeggeler B et al. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J 1993; **1**:57–60.
2. Hardeland R, Pandi-Perumal SR. Melatonin, a potent agent in antioxidative defense: actions as a natural food constituent, gastrointestinal factor, drug and prodrug. Nutr Metab (Lond) 2005; **2**:1–15.
3. Hardeland R, Cardinali DP, Srinivasan V et al. Melatonin-A pleiotropic, orchestrating regulator molecule. Prog Neurobiol 2011; **93**:350–384.

4. Galano A, Tan DX, Reiter RJ. Melatonin as a natural ally against oxidative stress: a physicochemical examination. J Pineal Res 2011; **51**:1–16.
5. Park S, Lee DE, Jang H et al. Melatonin-rich transgenic rice plants exhibit resistance to herbicide-induced oxidative stress. J Pineal Res 2013. doi: 10.1111/j.1600-079X.2012.01029.x. [Epub ahead of print].
6. Liu Y, Zhang L, Zhang H et al. Exogenous melatonin modulates apoptosis in the mouse brain induced by high-LET carbon ion irradiation. J Pineal Res 2012; **52**:47–56.
7. Tal O, Haim A, Harel O et al. Melatonin as an antioxidant and its semi-lunar rhythm in green macroalga Ulva sp. J Exp Bot 2011; **62**:1903–1910.
8. Reiter RJ, Tan DX, Fuentes-Broto L. Melatonin: a multitasking molecule. Prog Brain Res 2010; **181**:127–151.
9. Reiter RJ, Paredes SD, Manchester LC et al. Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit Rev Biochem Mol Biol 2009; **44**:175–200.
10. Reiter RJ, Pablos MI, Agapito TT et al. Melatonin in the context of the free radical theory of aging. Ann N Y Acad Sci 1996; **786**:362–378.
11. Kilic U, Yilmaz B, Ugur M et al. Evidence that membrane-bound G protein-coupled melatonin receptors MT1 and MT2 are not involved in the neuroprotective effects of melatonin in focal cerebral ischemia. J Pineal Res 2012; **52**:228–235.
12. Hardeland R, Poeggeler B. Non-vertebrate melatonin. J Pineal Res 2003; **34**:233–241.
13. Escames G, Ozturk G, Bano-Otalora B et al. Exercise and melatonin in humans: reciprocal benefits. J Pineal Res 2012; **52**:1–11.
14. Tan DX, Hardeland R, Manchester LC et al. The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. Biol Rev Camb Philos Soc 2010; **85**:607–623.
15. Hardeland R, Madrid JA, Tan DX et al. Melatonin, the circadian multioscillator system and health: the need for detailed analyses of peripheral melatonin signaling. J Pineal Res 2012; **52**:139–166.
16. Blask DE, Hill SM, Dauchy RT et al. Circadian regulation of molecular, dietary, and metabolic signaling mechanisms of human breast cancer growth by the nocturnal melatonin signal and the consequences of its disruption by light at night. J Pineal Res 2011; **51**:259–269.
17. Motilva V, Garcia-Maurino S, Talero E et al. New paradigms in chronic intestinal inflammation and colon cancer: role of melatonin. J Pineal Res 2011; **51**:44–60.
18. Tan DX, Manchester LC, Terron MP et al. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007; **42**:28–42.
19. Stehle JH, Saade A, Rawashdeh O et al. A survey of molecular details in the human pineal gland in the light of phylogeny, structure, function and chronobiological diseases. J Pineal Res 2011; **51**:17–43.
20. Migliori ML, Romanowski A, Simonetta SH et al. Daily variation in melatonin synthesis and arylalkylamine N-acetyltransferase activity in the nematode *Caenorhabditis elegans*. J Pineal Res 2012; **53**:38–46.
21. Yilmaz T, Celebi S, Kukner AS. The protective effects of melatonin, vitamin E and octreotide on retinal edema during ischemia-reperfusion in the guinea pig retina. Eur J Ophthalmol 2002; **12**:443–449.
22. Yilmaz S, Yilmaz E. Effects of melatonin and vitamin E on oxidative-antioxidative status in rats exposed to irradiation. Toxicology 2006; **222**:1–7.
23. Wahab MH, Akoul ES, Abdel-Aziz AA. Modulatory effects of melatonin and vitamin E on doxorubicin-induced cardiotoxicity in Ehrlich ascites carcinoma-bearing mice. Tumori 2000; **86**:157–162.
24. Tuzcu M, Baydas G. Effect of melatonin and vitamin E on diabetes-induced learning and memory impairment in rats. Eur J Pharmacol 2006; **537**:106–110.
25. Sharma S, Haldar C. Comparative effect of melatonin and vitamin E on phenylhydrazine-induced toxicity in the spleen of *Funambulus pennanti*. Environ Toxicol 2009; **24**:1–9.
26. Sonmez MF, Narin F, Akkus D et al. Melatonin and vitamin C ameliorate alcohol-induced oxidative stress and eNOS expression in rat kidney. Ren Fail 2012; **34**:480–486.
27. Sener G, Sehirli AO, Ayanoglu-Dulger G. Protective effects of melatonin, vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: a comparative study. J Pineal Res 2003; **35**:61–68.
28. Tunez I, Munoz MC, Medina FJ et al. Comparison of melatonin, vitamin E and L-carnitine in the treatment of neuro- and hepatotoxicity induced by thioacetamide. Cell Biochem Funct 2007; **25**:119–127.
29. Tan DX, Manchester LC, Reiter RJ et al. Significance of melatonin in antioxidative defense system: reactions and products. Biol Signals Recept 2000; **9**:137–159.
30. Ressmeyer AR, Mayo JC, Zelosko V et al. Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. Redox Rep 2003; **8**:205–213.
31. Lopez-Burillo S, Tan DX, Rodriguez-Gallego V et al. Melatonin and its derivatives cyclic 3-hydroxymelatonin, N^1-acetyl-N^2-formyl-5methoxykynuramine and 6-hydroxymelatonin reduce oxidative DNA damage induced by Fenton reagents. J Pineal Res 2003; **34**:178–184.
32. Catala A. The ability of melatonin to counteract lipid peroxidation in biological membranes. Curr Mol Med 2007; **7**:638–649.
33. Shida CS, Castrucci AM, Lamy-Freund MT. High melatonin solubility in aqueous medium. J Pineal Res 1994; **16**:198–201.
34. Venegas C, Garcia JA, Escames G et al. Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. J Pineal Res 2012; **52**:217–227.
35. Tan DX, Manchester LC, Sainz RM et al. Interactions between melatonin and nicotinamide nucleotide: NADH preservation in cells and in cell-free systems by melatonin. J Pineal Res 2005; **39**:185–194.
36. Mahal HS, Sharma HS, Mukherjee T. Antioxidant properties of melatonin: a pulse radiolysis study. Free Radic Biol Med 1999; **26**:557–565.
37. Tan DX, Manchester LC, Sainz R et al. Antioxidant strategies in protection against neurodegenerative disorders. Expert Opin Ther Pat 2003; **13**:1513–1543.
38. Barsacchi R, Kusmic C, Damiani E et al. Vitamin E consumption induced by oxidative stress in red blood cells is enhanced by melatonin and reduced by N-acetylserotonin. Free Radic Biol Med 1998; **24**:1187–1192.
39. Xu XD, Sun Y, Guo XQ et al. Effects of exogenous melatonin on ascorbate metabolism system in cucumber

seedlings under high temperature stress. Ying Yong Sheng Tai Xue Bao 2010; **21**:2580–2586.
40. Wang Y-J. Genetic transformation of *Nicotiana tabacum* L. and *Hypericum perforatum* L. by *Agrobacterium tumefaciens* carrying melatonin synthetase gene and enhancement of antioxidative capacity in transgenic plants. PhD Dissertation, College of Life Science, Northwest University, Xi'an, China, 2008.
41. Wang P, Yin L, Liang D et al. Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle. J Pineal Res 2012; **53**:11–20.
42. Marchetti C, Sidahmed-Adrar N, Collin F et al. Melatonin protects PLPC liposomes and LDL towards radical-induced oxidation. J Pineal Res 2011; **51**:286–296.
43. Gitto E, Tan DX, Reiter RJ et al. Individual and synergistic antioxidative actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferrioxamine (desferoxamine) in rat liver homogenates. J Pharm Pharmacol 2001; **53**:1393–1401.
44. Tan DX, Hardeland R, Manchester LC et al. Mechanistic and comparative studies of melatonin and classic antioxidants in terms of their interactions with the ABTS cation radical. J Pineal Res 2003; **34**:249–259.
45. Sahin N, Onderci M, Sahin K et al. Ascorbic acid and melatonin reduce heat-induced performance inhibition and oxidative stress in Japanese quails. Br Poult Sci 2004; **45**:116–122.
46. Morreale M, Livrea MA. Synergistic effect of glycolic acid on the antioxidant activity of alpha-tocopherol and melatonin in lipid bilayers and in human skin homogenates. Biochem Mol Biol Int 1997; **42**:1093–1102.
47. Lopez-Burillo S, Tan DX, Mayo JC et al. Melatonin, xanthurenic acid, resveratrol, EGCG, vitamin C and alpha-lipoic acid differentially reduce oxidative DNA damage induced by Fenton reagents: a study of their individual and synergistic actions. J Pineal Res 2003; **34**:269–277.
48. Zaouali MA, Reiter RJ, Padrissa-Altes S et al. Melatonin protects steatotic and nonsteatotic liver grafts against cold ischemia and reperfusion injury. J Pineal Res 2011; **50**:213–221.
49. Watanabe K, Hamada F, Wakatsuki A et al. Prophylactic administration of melatonin to the mother throughout pregnancy can protect against oxidative cerebral damage in neonatal rats. J Matern Fetal Neonatal Med 2012; **25**:1254–1259.
50. Zou LY, Liu SR, Li G et al. Melatonin reduced volume of cerebral infarct induced by photothrombosis in wild-type mice, not in cyclooxygenase-1 gene knockout mice. Conf Proc IEEE Eng Med Biol Soc 2004; **7**:4748–4750.
51. Wang WZ, Fang XH, Stephenson LL et al. Melatonin attenuates I/R-induced mitochondrial dysfunction in skeletal muscle. J Surg Res 2011; **171**:108–113.
52. Szarszoi O, Asemu G, Vanecek J et al. Effects of melatonin on ischemia and reperfusion injury of the rat heart. Cardiovasc Drugs Ther 2001; **15**:251–257.
53. Tai SH, Hung YC, Lee EJ et al. Melatonin protects against transient focal cerebral ischemia in both reproductively active and estrogen-deficient female rats: the impact of circulating estrogen on its hormetic dose-response. J Pineal Res 2011; **50**:292–303.
54. Kang JW, Lee SM. Melatonin inhibits type 1 interferon signaling of toll-like receptor 4 via heme oxygenase-1 induction in hepatic ischemia/reperfusion. J Pineal Res 2012; **53**:67–76.
55. Koh PO. Melatonin attenuates decrease of protein phosphatase 2A subunit B in ischemic brain injury. J Pineal Res 2012; **52**:57–61.
56. Sewerynek E, Reiter RJ, Melchiorri D et al. Oxidative damage in the liver induced by ischemia-reperfusion: protection by melatonin. Hepatogastroenterology 1996; **43**:898–905.
57. Zavodnik IB, Lapshina EA, Cheshchevik VT et al. Melatonin and succinate reduce rat liver mitochondrial dysfunction in diabetes. J Physiol Pharmacol 2011; **62**:421–427.
58. Yavuz O, Cam M, Bukan N et al. Protective effect of melatonin on beta-cell damage in streptozotocin-induced diabetes in rats. Acta Histochem 2003; **105**:261–266.
59. Bonnefond A, Clement N, Fawcett K et al. Rare MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. Nat Genet 2012; **44**:297–301.
60. Contreras-Alcantara S, Baba K, Tosini G. Removal of melatonin receptor type 1 induces insulin resistance in the mouse. Obesity (Silver Spring) 2010; **18**:1861–1863.
61. Sudnikovich EJ, Maksimchik YZ, Zabrodskaya SV et al. Melatonin attenuates metabolic disorders due to streptozotocin-induced diabetes in rats. Eur J Pharmacol 2007; **569**:180–187.
62. Wang X, Sirianni A, Pei Z et al. The melatonin MT1 receptor axis modulates mutant Huntingtin-mediated toxicity. J Neurosci 2011; **31**:14496–14507.
63. Tapias V, Cannon JR, Greenamyre JT. Melatonin treatment potentiates neurodegeneration in a rat rotenone Parkinson's disease model. J Neurosci Res 2010; **88**:420–427.
64. Singhal NK, Srivastava G, Agrawal S et al. Melatonin as a neuroprotective agent in the rodent models of Parkinson's disease: is it all set to irrefutable clinical translation? Mol Neurobiol 2012; **45**:186–199.
65. Rosales-Corral SA, Acuna-Castroviejo D, Coto-Montes A et al. Alzheimer's disease: pathological mechanisms and the beneficial role of melatonin. J Pineal Res 2012; **52**:167–202.
66. Patki G, Lau YS. Melatonin protects against neurobehavioral and mitochondrial deficits in a chronic mouse model of Parkinson's disease. Pharmacol Biochem Behav 2011; **99**:704–711.
67. Tan DX, Poeggeler B, Reiter RJ et al. The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo. Cancer Lett 1993; **70**:65–71.
68. Tan DX, Reiter RJ, Chen LD et al. Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. Carcinogenesis 1994; **15**:215–218.
69. Tas U, Ogeturk M, Meydan S et al. Hepatotoxic activity of toluene inhalation and protective role of melatonin. Toxicol Ind Health 2011; **27**:465–473.
70. Wang H, Xu DX, Lv JW et al. Melatonin attenuates lipopolysaccharide (LPS)-induced apoptotic liver damage in D-galactosamine-sensitized mice. Toxicology 2007; **237**:49–57.
71. Wang H, Wei W, Wang NP et al. Melatonin ameliorates carbon tetrachloride-induced hepatic fibrogenesis in rats via inhibition of oxidative stress. Life Sci 2005; **77**:1902–1915.
72. Tunon MJ, Miguel BS, Crespo I et al. Melatonin attenuates apoptotic liver damage in fulminant hepatic failure

induced by the rabbit hemorrhagic disease virus. J Pineal Res 2011; **50**:38–45.
73. Nickkholgh A, Schneider H, Sobirey M et al. The use of high-dose melatonin in liver resection is safe: first clinical experience. J Pineal Res 2011; **50**:381–388.
74. Chahbouni M, Escames G, Lopez LC et al. Melatonin treatment counteracts the hyperoxidative status in erythrocytes of patients suffering from Duchenne muscular dystrophy. Clin Biochem 2011; **44**:853–858.
75. Piechota A, Lipinska S, Szemraj J et al. Long-term melatonin administration enhances the antioxidant potential of human plasma maintained after discontinuation of the treatment. Gen Physiol Biophys 2010; **29**:144–150.
76. Velkov ZA, Velkov Y, Galunska BT et al. Melatonin: Quantum-chemical and biochemical investigation of antioxidant activity. Eur J Med Chem 2009; **44**:2834–3839.
77. Kedziora-Kornatowska K, Szewczyk-Golec K, Czuczejko J et al. Effect of melatonin on the oxidative stress in erythrocytes of healthy young and elderly subjects. J Pineal Res 2007; **42**:153–158.
78. Tan DX, Hardeland R, Manchester LC et al. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. J Exp Bot 2012; **63**:577–597.
79. Tan DX, Manchester LC, Di Mascio P et al. Novel rhythms of N1-acetyl-N2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation. FASEB J 2007; **21**:1724–1729.
80. Reiter RJ, Tan DX, Manchester LC et al. Melatonin reduces oxidant damage and promotes mitochondrial respiration: implications for aging. Ann N Y Acad Sci 2002; **959**:238–250.
81. Ozturk G, Akbulut KG, Guney S et al. Age-related changes in the rat brain mitochondrial antioxidative enzyme ratios: Modulation by melatonin. Exp Gerontol 2012; **47**:706–711.
82. Acuna-Castroviejo D, Lopez LC, Escames G et al. Melatonin-mitochondria Interplay in health and disease. Curr Top Med Chem 2011; **11**:221–240.
83. Acuna-Castroviejo D, Carretero M, Doerrier C et al. Melatonin protects lung mitochondria from aging. Age (Dordr) 2012; **34**:681–692.
84. Song N, Kim AJ, Kim HJ et al. Melatonin suppresses doxorubicin-induced premature senescence of A549 lung cancer cells by ameliorating mitochondrial dysfunction. J Pineal Res 2012; **53**:335–343.
85. Zhang N, Zhao B, Zhang HJ et al. Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus L.*). J Pineal Res 2013. doi: 10.1111/j.1600-079X.2012.01015.x. [Epub ahead of print].
86. Kang K, Lee K, Park S et al. Enhanced production of melatonin by ectopic overexpression of human serotonin N-acetyltransferase plays a role in cold resistance in transgenic rice seedlings. J Pineal Res 2010; **49**:176–182.
87. Arnao MB, Hernandez-Ruiz J. Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. J Pineal Res 2009; **46**:58–63.
88. Bergamini CM, Gambetti S, Dondi A et al. Oxygen, reactive oxygen species and tissue damage. Curr Pharm Des 2004; **10**:1611–1626.
89. Lenaz G. Mitochondria and reactive oxygen species. Which role in physiology and pathology? Adv Exp Med Biol 2012; **942**:93–136.
90. Hardeland R, Coto-Montes A, Poeggeler B. Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. Chronobiol Int 2003; **20**:921–962.
91. Acuna-Castroviejo D, Martin M, Macias M et al. Melatonin, mitochondria, and cellular bioenergetics. J Pineal Res 2001; **30**:65–74.
92. Martin M, Macias M, Leon J et al. Melatonin increases the activity of the oxidative phosphorylation enzymes and the production of ATP in rat brain and liver mitochondria. Int J Biochem Cell Biol 2002; **34**:348–357.
93. Martin M, Macias M, Escames G et al. Melatonin-induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. J Pineal Res 2000; **28**:242–248.
94. Petrosillo G, Moro N, Ruggiero FM et al. Melatonin inhibits cardiolipin peroxidation in mitochondria and prevents the mitochondrial permeability transition and cytochrome c release. Free Radic Biol Med 2009; **47**:969–974.
95. Jou MJ. Melatonin preserves the transient mitochondrial permeability transition for protection during mitochondrial Ca(2+) stress in astrocyte. J Pineal Res 2011; **50**:427–435.
96. Andrabi SA, Sayeed I, Siemen D et al. Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism responsible for anti-apoptotic effects of melatonin. FASEB J 2004; **18**:869–871.
97. Lopez A, Garcia JA, Escames G et al. Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. J Pineal Res 2009; **46**:188–198.
98. Arese M, Magnifico MC, Mastronicola D et al. Nanomolar melatonin enhances nNOS expression and controls HaCaT-cells bioenergetics. IUBMB Life 2012; **64**:251–258.
99. Petrosillo G, Moro N, Paradies V et al. Increased susceptibility to Ca(2+)-induced permeability transition and to cytochrome c release in rat heart mitochondria with aging: Effect of melatonin. J Pineal Res 2010; **48**:340–346.
100. Petrosillo G, Colantuono G, Moro N et al. Melatonin protects against heart ischemia-reperfusion injury by inhibiting mitochondrial permeability transition pore opening. Am J Physiol Heart Circ Physiol 2009; **297**:H1487–H1493.
101. Mei Q, Diao L, Xu JM et al. A protective effect of melatonin on intestinal permeability is induced by diclofenac via regulation of mitochondrial function in mice. Acta Pharmacol Sin 2011; **32**:495–502.
102. Hibaoui Y, Roulet E, Ruegg UT. Melatonin prevents oxidative stress-mediated mitochondrial permeability transition and death in skeletal muscle cells. J Pineal Res 2009; **47**:238–252.
103. Fischer TW, Zmijewski MA, Wortsman J et al. Melatonin maintains mitochondrial membrane potential and attenuates activation of initiator (casp-9) and effector caspases (casp-3/casp-7) and PARP in UVR-exposed HaCaT keratinocytes. J Pineal Res 2008; **44**:397–407.
104. Tan DX, Manchester LC, Fuentes-Broto L et al. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obes Rev 2011; **12**:167–188.
105. Reiter RJ, Tan DX, Korkmaz A et al. Obesity and metabolic syndrome: Association with chronodisruption, sleep

deprivation, and melatonin suppression. Ann Med 2012; **44**:564–577.
106. Rastmanesh R, De Bruin PF. Potential of melatonin for the treatment or prevention of obesity: an urgent need to include weight reduction as a secondary outcome in clinical trials of melatonin in obese patients with sleep disorders. Contemp Clin Trials 2012; **33**:574–575.
107. Garcia-Macia M, Vega-Naredo I, De Gonzalo-Calvo D et al. Melatonin induces neural SOD2 expression independent of the NF-kappaB pathway and improves the mitochondrial population and function in old mice. J Pineal Res 2011; **50**:54–63.
108. Okatani Y, Wakatsuki A, Reiter RJ et al. Acutely administered melatonin restores hepatic mitochondrial physiology in old mice. Int J Biochem Cell Biol 2003; **35**:367–375.
109. Okatani Y, Wakatsuki A, Reiter RJ et al. Hepatic mitochondrial dysfunction in senescence-accelerated mice: correction by long-term, orally administered physiological levels of melatonin. J Pineal Res 2002; **33**:127–133.
110. Dragicevic N, Delic V, Cao C et al. Caffeine increases mitochondrial function and blocks melatonin signaling to mitochondria in Alzheimer's mice and cells. Neuropharmacology 2012; **63**:1368–1379.
111. Martin M, Macias M, Escames G et al. Melatonin but not vitamins C and E maintains glutathione homeostasis in t-butyl hydroperoxide-induced mitochondrial oxidative stress. FASEB J 2000; **14**:1677–1679.
112. Semak I, Naumova M, Korik E et al. A novel metabolic pathway of melatonin: oxidation by cytochrome C. Biochemistry 2005; **44**:9300–9307.
113. Chyan YJ, Poeggeler B, Omar RA et al. Potent neuroprotective properties against the Alzheimer beta-amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid. J Biol Chem 1999; **274**:21937–21942.
114. Roberts JE, Hu DN, Wishart JF. Pulse radiolysis studies of melatonin and chloromelatonin. J Photochem Photobiol, B 1998; **42**:125–132.
115. Solis-Munoz P, Solis-Herruzo JA, Fernandez-Moreira D et al. Melatonin improves mitochondrial respiratory chain activity and liver morphology in ob/ob mice. J Pineal Res 2011; **51**:113–123.
116. Carretero M, Escames G, Lopez LC et al. Long-term melatonin administration protects brain mitochondria from aging. J Pineal Res 2009; **47**:200–209.
117. Garcia-Mesa Y, Gimenez-Llort L, Lopez LC et al. Melatonin plus physical exercise are highly neuroprotective in the 3xTg-AD mouse. Neurobiol Aging 2012; **33**:1124.e13–1124.e29.
118. Xiong N, Long X, Xiong J et al. Mitochondrial complex I inhibitor rotenone-induced toxicity and its potential mechanisms in Parkinson's disease models. Crit Rev Toxicol 2012; **42**:613–632.
119. Choi EM. Honokiol protects osteoblastic MC3T3-E1 cells against antimycin A-induced cytotoxicity. Inflamm Res 2011; **60**:1005–1012.
120. Henderson JR, Swalwell H, Boulton S et al. Direct, real-time monitoring of superoxide generation in isolated mitochondria. Free Radic Res 2009; **43**:796–802.
121. Zhou H, Chen J, Lu X et al. Melatonin protects against rotenone-induced cell injury via inhibition of Omi and Bax-mediated autophagy in Hela cells. J Pineal Res 2012; **52**: 120–127.
122. Saravanan KS, Sindhu KM, Mohanakumar KP. Melatonin protects against rotenone-induced oxidative stress in a hemiparkinsonian rat model. J Pineal Res 2007; **42**:247–253.
123. Zhang HM, Zhang Y, Zhang BX. The role of mitochondrial complex III in melatonin-induced ROS production in cultured mesangial cells. J Pineal Res 2011; **50**:78–82.
124. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2005; **2**:85–93.
125. Mailloux RJ, Harper ME. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. Free Radic Biol Med 2011; **51**:1106–1115.
126. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 2005; **27**:119–130.
127. Jou MJ, Peng TI, Yu PZ et al. Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis. J Pineal Res 2007; **43**:389–403.
128. Mayo JC, Sainz RM, Antoli I et al. Melatonin regulation of antioxidant enzyme gene expression. Cell Mol Life Sci 2002; **59**:1706–1713.
129. Inarrea P, Casanova A, Alava MA et al. Melatonin and steroid hormones activate intermembrane Cu,Zn-superoxide dismutase by means of mitochondrial cytochrome P450. Free Radic Biol Med 2011; **50**:1575–1581.
130. Zwirska-Korczala K, Jochem J, Adamczyk-Sowa M et al. Influence of melatonin on cell proliferation, antioxidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes–an in vitro study. J Physiol Pharmacol 2005; **56** (Suppl 6):91–99.
131. Wu CC, Lu KC, Lin GJ et al. Melatonin enhances endogenous heme oxygenase-1 and represses immune responses to ameliorate experimental murine membranous nephropathy. J Pineal Res 2012; **52**:460–469.
132. Crespo E, Macías M, Pozo D et al. Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction syndrome in rats. FASEB J 1999; **13**:1537–1546.
133. Escames G, León J, Macías M et al. Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. FASEB J 2003; **17**:932–934.
134. Wang H, Li L, Zhao M et al. Melatonin alleviates lipopolysaccharide-induced placental cellular stress response in mice. J Pineal Res 2011; **50**:418–426.
135. Park S, Lee SK, Park K et al. Beneficial effects of endogenous and exogenous melatonin on neural reconstruction and functional recovery in an animal model of spinal cord injury. J Pineal Res 2012; **52**:107–119.
136. Deng WG, Tang ST, Tseng HP et al. Melatonin suppresses macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression by inhibiting p52 acetylation and binding. Blood 2006; **108**:518–524.
137. Tahan G, Gramignoli R, Marongiu F et al. Melatonin expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic-acid-induced colitis in rats. Dig Dis Sci 2011; **56**:715–720.
138. Krakowski G, Cieciura L. Ultrastructural studies on the pinealocyte mitochondria during the daytime and at night. J Pineal Res 1985; **2**:315–324.

139. Cieciura L, Krakowski G. Ultrastructural studies on the mitochondria of the pinealocyte under conditions of persistent lighting–L24. Folia Histochem Cytobiol 1986; **24**:33–37.
140. Aarseth JJ, Stokkan KA. Quantitative differences in the pineal ultrastructure of perinatal and adult harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*). J Pineal Res 2003; **35**:188–195.
141. Karasek M, Stankov B, Lucini V et al. Comparison of the rat pinealocyte ultrastructure with melatonin concentrations during daytime and at night. J Pineal Res 1990; **9**: 251–257.
142. Karasek M, Zielinska A, Marek K et al. Effect of superior cervical ganglionectomy on the ultrastructure of pinealocytes in the Djungarian hamster (*Phodopus sungorus*): quantitative study. Neuro Endocrinol Lett 2002; **23**:443–446.
143. Nowicki M, Lewczuk B, Przybylska-Gornowicz B. Influence of 4-day long treatment with vasoactive intestinal peptide on ultrastructure and function of the rat pinealocytes in organ culture. Folia Histochem Cytobiol 2002; **40**:9–16.
144. Packer L. Metabolic and structural states of mitochondria. I. Regulation by adenosine diphosphate. J Biol Chem 1960; **235**:242–249.
145. Yuan H, Tang F, Pang SF. Binding characteristics, regional distribution and diurnal variation of [125I]-iodomelatonin binding sites in the chicken brain. J Pineal Res 1990; **9**:179–191.
146. Poon AM, Pang SF. 2[125I]iodomelatonin binding sites in spleens of guinea pigs. Life Sci 1992; **50**:1719–1726.
147. Kerenyi NA, Sotonyi P, Somogyi E. Localizing acetylserotonin transferase by electron microscopy. Histochemistry 1975; **46**:77–80.
148. Kerenyi NA, Balogh I, Somogyi E et al. Cytochemical investigation of acetyl-serotonin-transferase activity in the pineal gland. Cell Mol Biol Incl Cyto Enzymol 1979; **25**:259–262.
149. Blázquez E, López Gil A, Alvarez E, Muñoz Barragán L. Biochemical and ultrastructural approaches to the onset of the pineal melatonin rhythm in the rat. Neuroendocrinology 1989; **50**:500–505.
150. Clabough JW. Cytological aspects of pineal development in rats and hamsters. Am J Anat 1973; **137**:215–229.
151. Hirata F, Hayaishi O, Tokuyama T et al. In vitro and in vivo formation of two new metabolites of melatonin. J Biol Chem 1974; **249**:1311–1313.
152. Semak I, Korik E, Antonova M et al. Metabolism of melatonin by cytochrome P450s in rat liver mitochondria and microsomes. J Pineal Res 2008; **45**:515–523.
153. Sagan L. On the origin of mitosing cells. J Theor Biol 1967; **14**:255–274.
154. Esser C, Ahmadinejad N, Wiegand C et al. A genome phylogeny for mitochondria among alpha-proteobacteria and a predominantly eubacterial ancestry of yeast nuclear genes. Mol Biol Evol 2004; **21**:1643–1660.
155. Manchester LC, Poeggeler B, Alvares FL et al. Melatonin immunoreactivity in the photosynthetic prokaryote *Rhodospirillum rubrum*: implications for an ancient antioxidant system. Cell Mol Biol Res 1995; **41**: 391–395.
156. Tilden AR, Becker MA, Amma LL et al. Melatonin production in an aerobic photosynthetic bacterium: an evolutionarily early association with darkness. J Pineal Res 1997; **22**:102–106.
157. Fukumori Y, Watanabe K, Yamanaka T. Cytochrome aa3 from the aerobic photoheterotroph *Erythrobacter longus*: purification, and enzymatic and molecular features. J Biochem 1987; **102**:777–784.
158. Kang K, Lee K, Park S et al. Molecular cloning of rice serotonin N-acetyltransferase, the penultimate gene in plant melatonin biosynthesis. J Pineal Res 2013; doi: 10.1111/jpi.12011. [Epub ahead of print].
159. Awantang R, Tilden AR. Melatonin: Its Potential Application in Medicine and Its Evolution in Organisms. Macalester College Press, St Paul, 1998.
160. Hattori A, Wada M, Majima A et al. Detection of melatonin and synthesizing enzyme activities in cyanobacterium, *Spirulina platensis*. Proc Japan Soc Comp Endocrinol 1999; **14**:49.
161. Majima A, Hattori A, Wada M et al. Dynamic release of melatonin in cyanobacterium, *Spirulina platensis*. Proc Japan Soc Comp Endocrinol 1999; **14**:50.
162. Adams KL, Palmer JD. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. Mol Phylogenet Evol 2003; **29**:380–395.