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Review Article

Melanins: Skin Pigments and Much More—Types, Structural Models, Biological Functions, and Formation Routes

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This review presents a general view of all types of melanin in all types of organisms. Melanin is frequently considered just an animal cutaneous pigment and is treated separately from similar fungal or bacterial pigments. Similarities concerning the phenol precursors and common patterns in the formation routes are discussed. All melanins are formed in a first enzymatically-controlled phase, generally a phenolase, and a second phase characterized by an uncontrolled polymerization of the oxidized intermediates. In that second phase, quinones derived from phenol oxidation play a crucial role. Concerning functions, all melanins show a common feature, a protective role, but they are not merely photoprotective pigments against UV sunlight. In pathogenic microorganisms, melanization becomes a virulence factor since melanin protects microbial cells from defense mechanisms in the infected host. In turn, some melanins are formed in tissues where sunlight radiation is not a potential threat. Then, their redox, metal chelating, or free radical scavenging properties are more important than light absorption capacity. These pigments sometimes behave as a double-edged sword, and inhibition of melanogenesis is desirable in different cells. Melanin biochemistry is an active field of research from dermatological, biomedical, cosmetical, and microbiological points of view, as well as fruit technology.

1. Antecedents, Concept, Types, and Occurrence

Melanin is the generic name used to refer to perhaps the most ubiquitous, resistant, heterogeneous, and ancient pigments found in nature. Melanin appeared very early in most living kingdoms on the Earth. Thus, melanin has been recently found in very old fossils from dinosaurs, early birds, nonavian theropod species [1, 2], and primitive cephalopods [3]. These recent findings will probably make melanin a new biomarker in life evolution.

The name "melanin" comes from the ancient Greek *melanos*, meaning "dark," and, according to Borovansky [4], the term was probably first applied by the Swedish chemist Berzelius in 1840 to call a dark pigment extracted from eye membranes [5]. However, first references of human skin pigmentation and somehow to the existence of melanin without using the current name are very old. Pharaonic medicine in the Ebers Papyrus (1550 BC) described some diseases affecting skin color [6], and one of them was probably vitiligo, although that term appeared much later, derived from the Latin word "*vitellus*" meaning "veal" or pale pink skin [7]. The first relatively detailed written description on skin pigmentation in mankind came from Herodotus in Greece, who described the darker skin of Persians, Ethiopians, and Indians in relation with Greeks.

According to the Bible, the varieties of human color could have descended from Noah's three sons Shem, Japheth, and Ham (which means black). But that dark color of Ham and his descendants was attributed to the influence of hot water falling from the sky, until Aristotle attributed it to a prolonged burning sun. Aristotle's explanation is somehow the first link between sunlight and tanning or stimulation of cutaneous melanogenesis. To my knowledge, no record detailing any further scientific advance in skin or eye color is found during the middle age. In the modern era, there were a great number of anatomical descriptions by eminent scientists of those centuries, such as Malpighi in Naples and many others (see [7] for details). In those centuries, the most common idea in Europe and the Arabic civilization about the

origin of the dark pigment was that it was derived from the decomposition of hemoglobin. The first chemical analyses by Berzelius and others established very significant differences between melanin and hemoglobin, making it unlikely or ruling out such relationship.

Structurally, melanins are a group of complex pigments with a structure relative diverse and undefined. They have been defined in several ways during the last 50 years [8–10] but most of the proposed definitions present some small pitfalls as they are partial or uncompleted due to the difficulty of defining something with such a wide diversity in composition, color, size, occurrence, and functions. This is obvious since melanin can be found in all living kingdoms. A widespread and simple definition to include all types of melanin would be "*heterogeneous polymer derived by the oxidation of phenols and subsequent polymerization of intermediate phenols and their resulting quinones*."

To better comprehension of the melanin concept and structure, this definition should be followed by a classification of melanin in at least five main types according to the source: animal melanin, plant melanin, fungal melanin, bacterial melanin, and synthetic melanin. The phenolic units of the polymer and the properties of the oxidases needed for the polymer formation show significant differences. During the study and extraction of different melanins from these sources, particular names have appeared, mainly eumelanin, pheomelanin, neuromelanin, allomelanin, and pyomelanin, but there is not always a good correlation among the source and type of melanin.

2. Animal Melanins

Melanin is the main pigment responsible for the various pigmentations found in animal and human skin, hair, and eyes. As stated by Dr. Jablonski in her book "Skin: a natural story" [11], "*In the Homo sapiens, skin colors make up an exquisite palette, varying in almost imperceptible degrees from the palest ivories to the darkest browns*." This is mostly due to the melanin diversity and its genetic determinants [12–15], although other biomolecules such as carotenoids, hemoglobin, and nutritional factors can also contribute in a certain extension to the skin, hair, and eye tone [16–18]. Obviously, skin colors in the animal world are much richer than in human races. Basically, most of melanins are dark, from black to brown, but other melanins are reddish or yellowish [19]. According to that, animal melanins are divided in two large groups, eumelanin (eu = good) and pheomelanin (pheo = cloudy or dusky).

Both types are synthesized from the amino acid *L*-tyrosine with the participation of tyrosinase, the key enzyme of melanogenesis in animals and many microorganisms. Thus, *L*-tyrosine should be considered the main monophenol precursor of melanin (Figure 1(a)), although not the only one. Eumelanin provides primarily dark colors, from brown to black. Small amounts of eumelanin can give place to grey colors, as in human hair at mature age [20]. Eumelanin structure has been hampered, because it is not well defined and it is difficult to study due to its insolubility and resistance. Due to the unavailability of crystallized melanin, structure could not be ascertained by the most powerful classical physical methods such as X-ray diffraction. Alternatively, some solid-state NMR studies have also been carried out to explore hair and sepia melanin structure [21–23], but in general these attempts have offered rather limited information concerning the molecular structure of the polymer. Any chemical treatment to dissolve eumelanin alters its native structure and breaks the initial polymer in fragments, whereas enzymatic digestion is even relatively inefficient to eliminate the protein and lipid content of natural samples [23].

Chemical degradation may be usually performed by hot oxidation with permanganate or hydrogen peroxide in alkaline media, or other aggressive treatments. These methods and the chemical identification of a number of characteristic fragments have been developed during years by the group of Professor Ito in Nagoya [24–27]. The main fragments are the carboxylic pyrrolic acids PDCA, PTCA, and others and aminohydroxyphenylalanine (AHP) units. There are detailed and updated reviews about HPLC quantitation, structure of those fragments, and their utility as markers for qualitative and quantitative melanin determination [10, 27, 28].

Anyway, eumelanin from different sources is heterogeneous in size and in its fine chemical structure. Following the empirical formula ($C_8H_3O_2N$) suggested by Mason [29], there are still some data on websites giving a defined stoichiometric structure [30] where melanin, ID: 4884931, is defined with the molecular formula $C_{18}H_{10}O_4N_2$, an average mass of 318.3 for the minimal unit, and the systematic name 3,8-dimethyl-2,7-dihydrobenzo[1,7]isoindolo[6,5,4-cd]indole-4,5,9,10-tetrone, [30] Figure 2(a). As Mason's original proposal, these data should be considered as an empirical approximation. The natural occurrence of indole rings condensation assumed at the above tetrone model is very unlikely, although it has been sometimes proposed, as in the eumelanin pigment formed in the insect exocuticle [31].

According to most of the experimental data, the main units or building blocks are 5,6-dihydroxyindole units (DHI). Active positions for polymerization in this unit are 2, 3, 4, and 7 [25, 26, 32, 33]. Some of these units are carboxylated at position 2 (5,6-dihydroxyindole-2-carboxylic acid units, DHICA units). The ratio DHI/DHICA of the eumelanin depends on the dopachrome tautomerase activity and/or the presence of traces of metal ions in the media of synthesis [34, 35].

A model fragment of eumelanin is depicted at Figure 2(b). Covalent bonds 4 → 7 are the most abundant links among units, but a variety of other bonds involving positions 2 and 3 are also possible to incorporate DHI units. Position 2 is blocked at DHICA units, and position 3 is mostly inactivated to the electron withdrawing effect of the carboxyl group at position 2. Thus, DHICA units trend to linear polymer, but DHI units trend to branched and larger molecules. Uncycled units of *L*-dopa or even *L*-tyrosine or oxidized indolic units of 5,6-indole quinone (IQ) can also be incorporated in low proportion to the polymer during its formation (Figure 2(b)). Finally, some data also suggest the presence of carboxylated pyrrolic units in the polymer

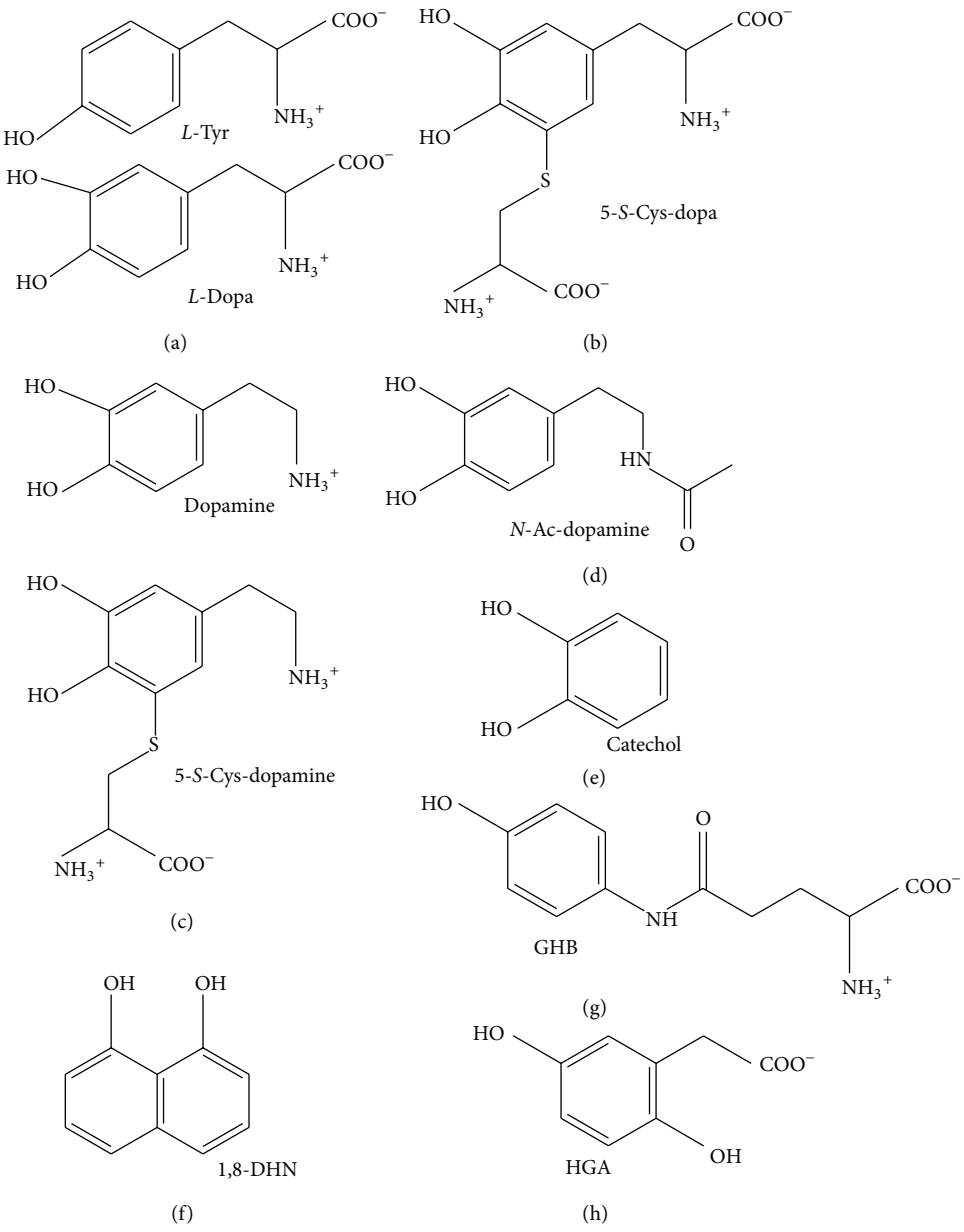

Figure 1: Structure of melanin precursors for the different types of melanin (in brackets). Only the most representative for every type is shown. (a) *L*-tyr and *L*-Dopa (eumelanin); (b) 5-cys-dopa (pheomelanin); (c) dopamine and 5-*S*-cys-dopamine (neuromelanin); (d) *N*-acetyl-dopamine (insect-melanin); (e) catechol (catechol-melanin, plants); (f) DHN, 1,8-dihydroxynaphthalene (DHN-melanin, fungi); (g) GHB, 4-glutaminyhydroxylbenzene (GHB-melanin, mushroom); (h) HGA, homogentisic acid (pyomelanin).

[36], although these units should mostly be formed during degradative treatments of analysis, as stated above. It is possible that the small presence of these units, if so, would be related to high oxidative conditions during the eumelanin formation and/or the aging of the polymer.

In addition, during melanin formation, some of these units can be conjugated with thiol or amino groups of amino acid side chains of peptides or proteins, forming melanoproteins [8, 37] of structure still more undefined. These conjugates were identified for the first time in sepiamelanin, where several different amino acids were identified in the melanin extracted from the cuttlefish ink [38], but melanoproteins were also described in other sources [39] due to the reactivity of quinones with chemical groups of the amino acid side chains [40]. This quinone reactivity makes it possible that a number of other nonphenolic substances present in the melanin-formation media can be incorporated to the polymer, such as thiouracil, an antithyroid drug used as a tumor marker for malignant melanoma for this reason [41, 42].

Pheomelanin is more treatable than eumelanin, as most of this pigment can be dissolved in alkali media. Pheomelanin produces yellowish or reddish colors, and it is found in relatively large quantities in red hair, freckles, and feathers

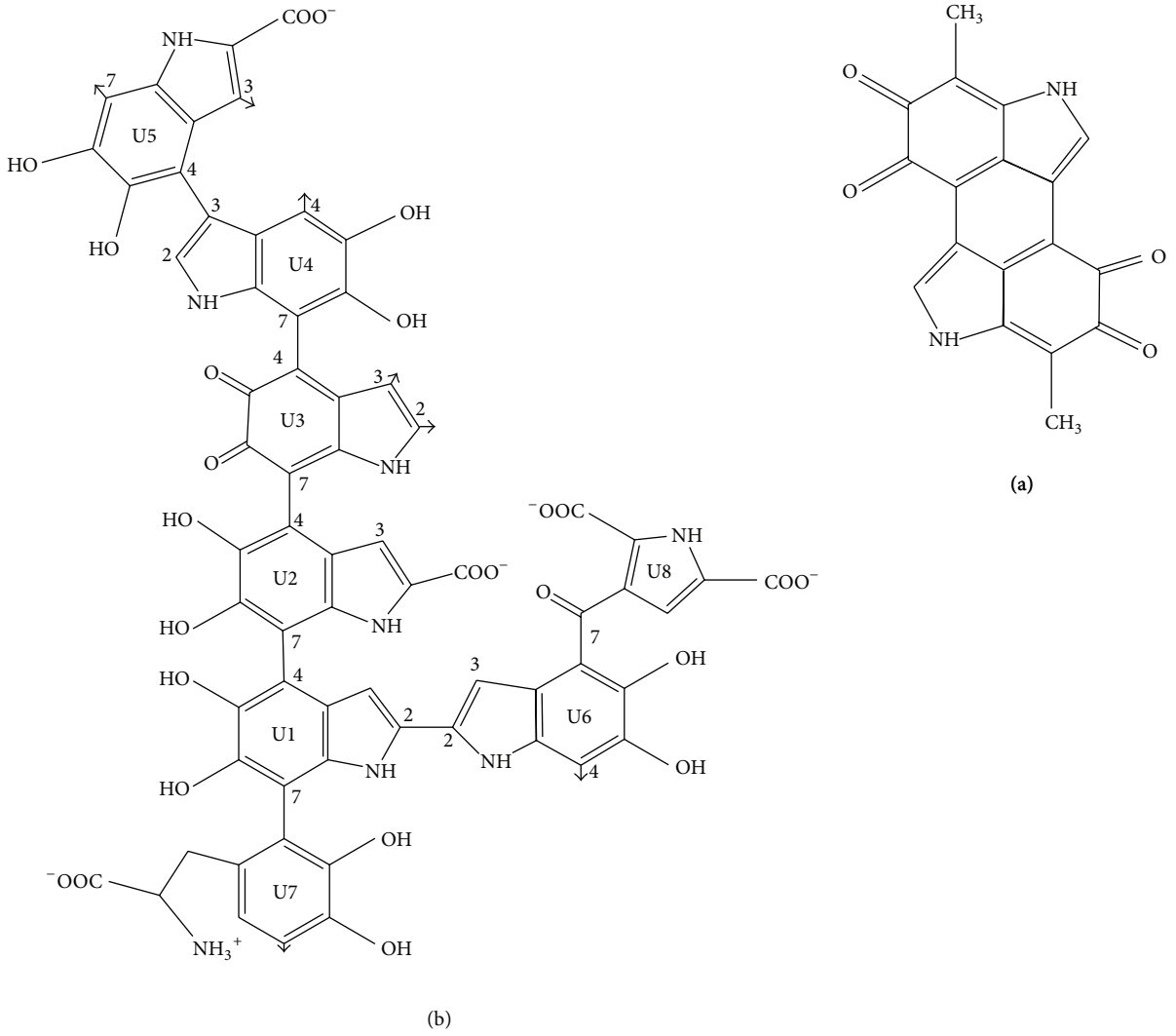

Figure 2: Model for eumelanin structure. This polymer is dark, from brown to black depending on the size and electronic conjugation. (a) The tetrone simplified model to account for empirical composition $C_{18}H_{10}O_4N_2$. (b) The classical model. Most units are indolic units. The most abundant units are DHI (5,6-dihydroxyindole, U1, U4, and U6) although some units of the 2-carboxylated analogue DHICA (5,6-dihydroxyindole-2-carboxylic acid, U2, and U5) are also found. The ratio DHI/DHICA depends on the dopachrome tautomerase activity and other conditions during the eumelanin formation. Oxidized units of IQ (5,6-indolequinone, U3), unaltered *L*-dopa units (U7), and carboxylated pyrroles due to partial degradation of indoles during the polymerization (U8) can also be incorporated during the polymerization. The units are linked by a variety of bonds involving positions 4, 7, 2, and 3. Arrows indicate some possible points for polymer growth.

of fowls and other birds [43, 44]. The main difference of eumelanin in chemical terms is the presence of sulfur. During the pheomelanogenesis, the amino acid *L*-cysteine, free or in compounds such as glutathione, is added to *L*-dopaquinone, the tyrosinase-catalyzed oxidized intermediate from *L*-tyrosine. Thus, *L*-dopaquinone is the branch point to form eu- or pheomelanin inside the melanocyte. The presence of sulfur in pheomelanins by addition of *L*-cysteine or glutathione was described by Prota [33, 43–45]. Cysteine can be added to several positions at the dopaquinone ring, but the 5-*S*-cysteinyldopa (Figure 1(b)) or 5-*S*-glutathionyldopa are the predominant isomers formed [32, 46].

Thus, pheomelanin consists of oligomers formed by sulfur-containing units, mostly benzothiazine and benzothiazole (Figure 3), instead of indole units [47, 48]. Benzothiazine units are first formed from cysteinyldopa, but its conversion to benzothiazole has been demonstrated [49]. The fine structure of pheomelanin is not well defined, and the presence of benzothiazolylthiazinodihydroisoquinoline rings has been proposed [47], but analog units with carboxylated indole rings (U1–U3 in Figure 3) would also be possible to occur taking into account that this moiety of the structure can be formed by direct cyclization of the *L*-tyrosine side chain. Mechanisms of benzothiazine (U1-U2) in benzothiazole (U4-U5) transformation, (dihydro)isoquinoline arrangement, and the possibility of indole units formation should await new structural studies of natural pheomelanin.

It has been believed that pheomelanin is specific of higher animals, mammals, or birds. Recently, using the current

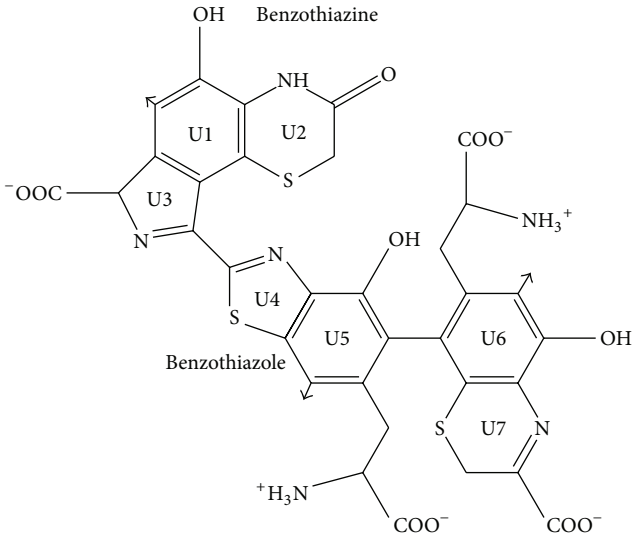

Figure 3: Model for pheomelanin structure. This pigment is fair color, from reddish to yellowish. It consists of two main units, benzothiazines (U1 + U2, U6 + U7) and benzothiazoles (U4 + U5). Both units are derived from the spontaneous addition of *L*-cysteine to *L*-dopaquinone during the melanin biosynthetic pathway and subsequent reactions and cyclization of cysteine residues originate the sulfur-containing rings. Side chains of dopaquinone remain opened or sometimes cycled (such as U3). These last cycles could also give place to dihydroisoquinoline units. Arrows indicate possible points for polymer growth.

chemical characterization procedures, it has been shown that pheomelanins can also occur in reptiles [50] but they are still very rare in lower organisms. Occurrence of pheomelanin was claimed in fungi [51] but the only criteria to propose that presence was the yellowish color of the fungal obtained pigments, without chemical analysis. This is not enough. Recently, new data about fungal pheomelanin have been published according to the color and also the presence of sulfur by chemical analysis [52], but the reported unit of those melanins is mostly indole suggesting that these are eumelanins rather than true pheomelanins.

In sum, the existence of fungal pheomelanin is still under discussion. The presence of small quantities of sulfur in pigments obtained from these microorganisms could be due to the addition of some thiol-containing compounds to a eumelanin-based polymer [32, 44]. In fact, the sepiamelanin described by Nicolaus is an accepted eumelanin model which has low amount of sulfur due to the addition of small amounts of protein during the polymerization, somehow acquiring melanoprotein nature [8]. On the other hand, granules of mixed melanin have been reported in neuromelanin (see later on). In the presence of small amounts of thiol compounds such as *L*-cysteine, 5-*S*-cysteinyldopas formation units are favored [23] and pheomelanin are formed in the core of the granule, but once the thiol groups are exhausted the polymerization goes on; eumelanin is formed on the surface of the granule [53].

Trichochromes also named pheochromes can be considered as a small variant of pheomelanin with a defined molecular weight [43, 44]. They are compounds derived from dimerization of two benzothiazines units formed from 5-cysteinyldopa. They were isolated from red hair and New Hampshire chicken feathers [8], although the amount is very small. There are several forms depending on the oxidation state of the thiazine ring and the presence of other groups on position 3 (Figure 4). They show brighter color in comparison to larger pheomelanins, which have a cloudy color according to the Greek meaning of the name.

Eumelanins are more abundant in human beings, especially in dark-skinned people. In Caucasian people, localized high cutaneous concentration of melanin can be found in moles, macules, nevi, or lentigos, whereas pheomelanin is found in freckles or nipples. Hyperpigmentation or hypopigmentation are important cutaneous disorders. Melanin can also occur pathologically, as in cutaneous melasma or in melanotic malignant melanoma. This type of cancer appeared by malignancy of cutaneous melanocytes, the melanin-forming cells. On the other hand, the total absence of pigmentation results in albinism and the partial absence in skin patched results in vitiligo and pielbaldism. In that way, melanin has cosmetic, social, and biomedical implications.

But mammalian melanins are not restricted to the skin and hair. There is an extracutaneous melanogenic system [54]. The pigment is also present in the tissue underlying the iris of the eye, eyespots, retinal epithelial pigment (REP) [55, 56], and the *stria vascularis* of the inner ear [57]. Thus, melanin is important for the correct functioning of the sight and hearing. The mysterious and intriguing connection between albinism, light-colored eyes, and deafness is well known many years ago, as Charles Darwin in his 1859 treatise "*On the Origin of Species*," wrote that "*cats which are entirely white and have blue eyes are generally deaf*." A partial comprehensive view of that connection is quite recent [58] although still uncompleted.

Melanin is also found in certain regions of the brain and adrenal gland of some mammals. Neuromelaninis a dark polymer pigment produced in specific populations of catecholaminergic neurons in the brain, mostly sited in the *substantia nigra* and in lower proportion in the *locus coeruleus*. It appears in greatest amounts in the human brain and in lesser amounts in some other nonhuman primates but is absent from the brain in many lower species. The high and specific presence of neuromelanin in human brain has increased the interest for this pigment and its role. It may play crucial roles in apoptosis, neurodegeneration, and the related Parkinson's disease [59, 60].

Neuromelanin is a mixture of pheomelanin and eumelanin, as they are derived from dopamine and 5-*S*-cysteinyldopamine, units identified by standard analysis methods [46] (Figure 1(c)). Thus, benzothiazine and indole units are found in this polymer [61–65]. According to Bush et al. [53], neuromelanin granules should have pheomelanin in the core and eumelanin in the surface, which is compatible with an occasional exhaustion of the glutathione or cysteine reduction system during neuromelanin formation [61].

In principle, neuromelanin seems to be a waste product of catecholamine metabolism, as the amount of cytoplasm within catecholamine neurons occupied by neuromelanin

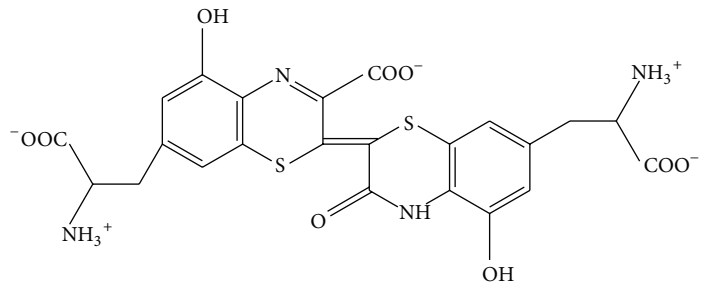

Figure 4: Trichochrome F: this is a typical trichochrome, but there are other variants from color orange to violet depending on the groups at position 2 and the possible double bond at position 3 of the thiazine ring. These molecules are dimers of two benzothiazine units, and they can be considered small pheomelanins with a well-defined structure.

was found to increase progressively with the brain's age [59]. However, other data confer that neuromelanin specific functions as dopaminergic neurons of the *substantia nigra* are critical factors to explain the vulnerability of this brain region to early and massive degeneration in Parkinson's disease [66].

However, the massive, early, and relatively circumscribed death of the dopaminergic neurons of the *substantia nigra* in Parkinson's disease has not yet been adequately explained. In spite of thousands of papers published on neuromelanin, the biological function remains mostly unknown, although human neuromelanin has been shown to efficiently bind metals such as iron, as well as other potentially toxic molecules [67]. It is well known that in systemic tissues melanin is a double-edged sword [68].

Finally, in mammals, there is ectopic formation of melanin even in some other tissues different from skin, eye, inner ear, and brain, as in the adipose tissue [69]. Tyrosinase RNA has been detected in samples of the latter tissue, although enzyme activity is low. Melanin in obsess seems to be functional and it may serve as a compensatory mechanism to use its oxidative damage-absorbing and anti-inflammatory properties in the adipose tissue [70].

Aside mammals, bird feathers contain the two types of melanin pigments, as well as a variety of carotenoids, although the relative amounts of each pigment differ with the avian species [71]. As in the mammalian hair, feather melanin is usually bound to the keratins forming the protein matrix of the hair and feather, respectively. In most cases, feather melanins are a mixture of eu- and pheomelanin, but the brightness and hue of the feather color does not anticipate the ratio [72]. Eumelanin and pheomelanin concentrations used to be significantly positively correlated in the feathers of females but not males [73], although the eumelanin-based coloration can be a sexually selected signal of resistance to the environmental oxidative stress [74] and other stressful events [75].

Melanin is also found in reptiles, amphibians, and fish. These animals can change color relatively rapidly to camouflage themselves against changing backgrounds [76], so that melanin is more versatile and offers hue due to the existence of chromatophores and iridophores in addition to melanocytes. In these species, the melanosomes and the subcellular organelles that synthesize and accumulate the melanin are under hormonal and neural control, and they may be rapidly and reversibly transported into certain skin regions due to the MCH, melanin concentrating hormone, which is much more active in these animals than in mammals [77]. Hormonal control of melanization by MSH (melanin stimulating hormone) is also important in these animals, as well as birds and mammals, but this point is out of the scope of this review. Melanins in these animals are generally eumelanin, although some report on the presence of pheomelanin in tortoise has been recently published [50]. These animals have an active extracutaneous pigmentary system, so that melanin is also found in blood phagocytic cells [78], peritoneum, liver, and kidney [79–81] although the role of the pigment in those tissues is not well understood.

As stated above, the ink in the cuttlefish contains a special type of melanin called sepiamelanin. Melanins also produce the black coloration in the ink of octopus, squid, and other cephalopods, forming a granule material dispersed in colorless plasma. This melanin is mostly eumelanin, as it is formed by an irregular heteropolymer of indole and carboxylated pyrrolic units with some associated protein [8, 82]. During melanin formation in these systems, there are clear evidences about the formation of hydrogen peroxide and free radicals during the polymerization process [83]. These free radicals are possibly responsible of the appearance of units such as the carboxylated pyrroles, derived from breaking the hexagonal ring of the indole units [36]. These carboxyl groups have special cation-chelating properties, making sepiamelanin a polymer with higher capacity to ion exchange than melanins from other sources. Anyway, sepiamelanin has been an excellent model for the study of eumelanin structure and properties, due to its easy extraction in significant amounts.

In insects, melanins are also crucial, although they show their own peculiar features. It is well known for many years that the exocuticleof insects is tanned by quinones derived by oxidation from catechols [84]. In that way, the exoskeleton is hardened to protect their soft bodies by a process called sclerotization, which is parallel and often accompanied with melanization or tanning [85, 86]. The main precursor for that process is *N*-acetyldopamine (Figure 1(d)), which is derived from the amino acid *L*-tyrosine, so this melanin should be considered as a special type of eumelanin [87]. Insect melanin

is not just and ornament. In birds and insects, it is used the term "*melanism,*" which is a costly mechanism enabling the protective trait in natural selection and it constitutes more than just a color change in the polymorphisms of a number of insect species [88, 89]. Insect melanogenesis is also considered as a process related to the insect innate immunity [90, 91] and insect hemostasis [92].

3. Plant Melanin

Plant metabolism is characterized by the principle of nitrogen economy, as this element is an important limiting factor for plant growing. According to that, the amino acid *L*-tyrosine is not used for the synthesis of plant melanin. Almost all these organisms, and some microorganisms, employ some phenol nitrogen-free precursors, such as catechols, dihydroxynaphthalenes or other types of dihydroxybenzenes to carry out this part of its secondary metabolism. In general, the obtained melanin is a polymer devoid of nitrogen and is generically named allomelanin (other melanins). In plants, the most common precursor is just catechol (Figure 1(e)); so the melanin formed is also named catechol-melanin [8, 32] and the enzymatic system involved in the synthesis is named catechol oxidase [93]. Tyrosine is a poor substrate of these catechol oxidases, but it should be clear that catechol-melanin is a subtype of allomelanin, and catechol oxidase which is the most frequent type of plant phenol oxidases [94], related to animal tyrosinases.

The color of allomelanin is always from dark brown to totally black, and its structure depends on the nature of the main unit oxidized. Some vegetables use just normal catechol, but others use different catecholic acids (such as caffeic, chlorogenic, protocatechuic, or gallic acids). According to that, the melanin pigment is a polymer derived from these catechols and the corresponding quinones formed by the action of catechol oxidases. The structure of these plant melanins has been poorly studied in the last years, and the efforts have been mostly directed to inhibiting it formation. In this regard, the main process studied has been the inhibition of the catechol oxidases and the isolation of natural inhibitors of melanin formation [95] to prevent the undesirable browning.

4. Fungal Melanin

As in birds, reptiles, and plants, microbial pigments are more than just melanin [96], but the relevance of this pigment and the great interest for fungal melanin is demonstrated by the publication of a great number of original papers, book chapters [97, 98], and many reviews on different aspects about the synthesis and function of this type of pigment [99–101]. One of the main reasons is the biotechnological importance of fungi and the prompt demonstration is that melanin play an essential role in the virulence of plant and human pathogenic fungi [102, 103]. The excellent and very complete review published by Nosanchuk and Casadevall [104] ten years ago is still very valuable for further details about the role of melanin in microbial pathogenesis, although there are other more recent review from the same group describing special aspects, such as the synthesis and assembly of fungal melanin [105].

Melanin pigments are very common in fungi, although melanogenesis is restricted to certain developmental stages on mycelium, sporulation, or defensive reactions to wounding. Fungal melanin is quite abundant and appears in the cell wall rather than in specialized subcellular organelles such as the animal melanosomes. Usually, in fungi and yeast, melanin precursors are secreted and then oxidized outside the cell wall [99].

The precursors and the nature of the corresponding melanin structure show variability. The first characterizations suggested catechol precursors to form allomelanin devoid of nitrogen by similarity to plant melanins [8, 32]. These initial studies were carried out to study pathogenic molds of rice plants (*Pyricularia oryzae*), and melanin was identified as a pathogenicity factor [106]. According to that nitrogen economy, *L*-tyrosine did not stimulate melanin formation.

However, the precursor identified was not catechol, but hydroxylated naphthalene-derived molecules (Figure 1(f)) [107]. In that way, a new subtype of allomelanins was described, the DHN-melanins (dihydroxynaphthalene melanin). This type of melanin is quite common in ascomycetous and some imperfect fungi [108] including nonmicroscopic fungi, as for instance in edible truffle [109]. The synthesis takes place by the pentaketide pathway. Scytalone is a key intermediate [104, 107]. However, *Aspergillus niger* and basidiomycetes did not form these hydroxynaphthalene substrates. Thus, many ascomycetes and some imperfect fungi appear to make DHN-melanin, whereas basidiomycetes and other imperfect fungi use alternative pathways [108].

Yeasts are unicellular fungi, and, therefore, some of them are also able to form melanin. For instance, the black fungus isolated from oak bark was identified as a member of the yeast-like genus *Phaeococcomyces*. It shows characteristics associated with basidiomycetous yeasts, but the pigment was identified as a DHN-melanin, which is more related to ascomycetes [110]. In some fungi, such as *Wangiella (Exophiala) dermatitidis*, the precursor could be an acetylated form of hydroxynaphthalene [111] which makes the polymerization of those units occur in a more regulated way. The pentaketide pathway is almost specific for fungi, although there are some reports on the existence of pentaketide synthases in plants and bacteria [112].

Basidiomycetes use a route for melanin synthesis more similar to animals. Most of this work has been done with mushrooms, *Agaricus bisporus* and others. These species contain a very active tyrosinase which is worldwide used as the commercial tyrosinase model for *in vitro* studies. In turn, from the historical point of view, other species of mushroom, *Russula nigricans*, were the source of one of the first enzymes (ferment) described in the initial era of the enzymology, by Bourquelot and Bertrand in 1895 [113]. In these basidiomycetes, melanization is not carried out in the mycelium and the pigment is a eumelanin formed from a particular precursor [114]. Melanin is formed from a benzoquinone obtained by the action of tyrosinase on the precursors, gamma-glutaminyl-4-hydroxybenzene (GHB, Figure 1(g)).

This synthesis of melanin could be considered specific of mushrooms and different from all other melanin pigments, but it has never been totally elucidated from the proposal in 1980 [115]. In any case, it seems that the amide nitrogen of the glutaminyl residue is incorporated to the melanin, but the glutamyl part is not incorporated to the mushroom pigment. Some of these particular intermediates in mushroom melanogenesis were proposed to have anticancer and melanotoxicity activity in mammals, although such effect has not been confirmed. A related metabolite, called agaritine β-N [γ-L (+)-glutaminyl]-4-hydroxymethylphenylhydrazine [116], seems to inhibit HIV proteases and it might be a good candidate for developing drugs for AIDS therapy [117], but it has also been reported that it prevents melanin formation [118].

A very interesting and studied case of fungal melanin is the neuropathogenic fungi *Cryptococcus neoformans* [119]. This basidiomycetous fungus infects the human brain and it contains a very active phenol oxidase able to oxidize the catecholamine neurotransmitters, dopamine, and norepinephrine to melanin polymers [120, 121]. As these pigments are formed in the brain after infection, a pathogenic neuromelanin is formed using brain compounds [122] and that increases the virulence of the infection. On the other hand, *C. neoformans* is also able to synthesize pyomelanin derived from a fungal metabolite, homogentisic acid (HGA, Figure 1(h)) [123]. This route gives place to a melanin in the form of "ghost-" like hydrophobic particles, fluorescent under a variety of wavelengths resistant to degradation by strong acids that could increase the virulence of this fungus.

The possibility of synthesizing two different types of melanin depending on the environmental conditions is not specific of *C. neoformans*. *Aspergillus fumigatus*, one of the most important airborne fungal pathogens of immunosuppressed humans, is able to produce DHN-melanin, which is predominantly present in the conidia. Its biosynthesis is again an important virulence determinant, but it is also able to synthesize pyomelanin starting from *L*-tyrosine through homogentisic acid [124]. Similarly, *Aspergillus nidulans* is able to form the DHN-melanin normally in ascomycetes and also DOPA-melanin [125].

5. Bacterial Melanin

Bacterial melanins are also common, and they have been observed in a great number of species as their cultures have black or dark brown colors. However, it is important to note that not all dark pigments in bacterial cultures are indeed melanin pigments. For instance, molasses from high-polluting industries are a suspension of dark color bacterial subproducts in wastewater, but they are not really melanin and should be treated in a different way for detoxification and discoloration [126].

Bacterial melanins were well first characterized in *Streptomyces*, as these species have been actively pursued in the search of antibiotics [127]. In fact, melanin formation was proposed as an appropriate technique for the classification of *Streptomyces* [128]. These species had been good models for the study of the enzyme tyrosinase, its structure, mechanism of catalysis, and regulation of its expression, rather than for the study the final melanin product [129, 130]. In *S. antibioticus* or *S. lividans*, the melanin pigment is rather an undesirable product since its formation hinders the isolation of the most important products from these species, antibiotics.

Similarly to *Streptomyces*, other species such as *Marinomonas mediterranea* [131, 132] and *Bacillus thuringiensis* [133, 134] contain active tyrosinases and they become heavily pigmented in the presence of *L*-tyrosine. All these bacterial melanins contain nitrogen, and their structure is similar to animal eumelanin. Melanin from *B. thuringiensis* has been deeply studied, as the melanized bacterial cell is protected from pesticides [135]. This melanin seems to be solubilized easier than others, and it has been proposed to be more convenient for alternative biotechnological uses. *Bacillus subtilis* synthesizes a brown pigment related to melanin for sporulation, but this process is catalyzed by a laccase-related protein named cotA [136].

Similarly to plants, there are also bacterial allomelanin from catecholic precursors. Thus, allomelanin formed in *Rhizobium* species is a mechanism for the detoxification of phenolic compounds which are accumulated in senescing nodules [137]. Certain bacterial species, such as *Azotobacter* [138], can also form melanin from catechols to create high respiration rates necessary for protection of the nitrogenase system in nitrogen fixation. Some bacteria are also able to carry out the pentaketide biosynthetic pathway leading to DHN-melanin, although the bacterial polyketide synthases have different features from those of the fungal and plant enzymes and possibly are able to incorporate other small aromatic metabolites to the melanin pigment [112]. Related to these last cases, there are a number of soil microorganisms (bacteria and fungi) which form dark polymers named humic acids which are acid-type phenolic polymers similar to allomelanin and also to lignin. All these polymers have highly similar spectra and physicochemical properties [139, 140] and are very resistant to decomposition and give long-life beneficial effects for soils, retaining ions, biomass, and water.

In agreement with the bacterial diversity, other species such as *S. marcescens* [141] form a brown pigment by oxidation and polymerization of intermediates of catabolic pathways. In that species, the melanin precursor is 3,4-dihydroxyphenylacetate. When the enzymes of this catabolic pathway are induced and the 3,4-dihydroxyphenylacetate-2,3-dioxygenase remained at low activity; the accumulation of the diphenolic intermediate leads to pigment production as a form to eliminate its excess. Nowadays, the polymer is considered allomelanin although the original paper doubted about the melanin-like nature of that product as it was formed without the involvement of a polyphenol oxidase [138]. Similar data were reported with some *Pseudomonas* [142, 143] due to a reduced 4-hydroxyphenylpyruvate dioxygenase activity, and the pigment is considered pyomelanin [144].

Regarding this concept, other bacterial species have no polyphenol oxidase but are able to form a yellowish pigment that is also named pyomelanin [8, 10, 124]. Its color resembles pheomelanin, but, in fact, these pyomelanins are different

from those formed by *Pseudomonas* as they are derived from the homogentisic acid, HGA-melanin (Figure 1(h)), an intermediate of the tyrosine catabolic pathway. As stated above, this type of melanin is also formed in some fungi. *Shewanella colweliana* [145] and *Vibrio cholerae* [146–148] synthesize pyomelanin in response to specific physiological conditions that are stressful to the bacteria. Under those conditions, there is accumulation of homogentisic acid because of the low activity of homogentisate dioxygenase. Thus, homogentisic acid is subsequently oxidized and an ochre-colored polymer is formed. More recently, this type of melanin has also been characterized in *Burkholderia cenocepacia,* a gram-negative opportunistic pathogen that can survive within phagocytic cells, and the synthesis of this melanin is a defense mechanism against the host cell [149], similarly to some pathogenic fungus, such as *Aspergillus fumigates* [124]. Moreover, pyomelanin from HGA has some relevance since the situation is comparable to the human alkaptonuria [147, 148], a rare disease due to mutations in the homogentisate dioxygenase human gene. This inactive enzyme leads to deposits of pyomelanin in the cartilage. It produces ochronosis, and it is confirmed by the rapid darkening of the urine of these patients under basic media.

6. Synthetic Melanin

Finally, these are also melanins formed by chemical oxidation from some diphenolic precursors. The most common ones are dopa-melanin and dopamine-melanin, which are easily formed by chemical oxidation of those precursors, generally using atmospheric oxygen or hydrogen peroxide in basic media [9, 24, 25]. They are used as model melanin for biophysical studies and other applications of eumelanins. They are totally devoid of protein, as they are formed in the absence of enzyme or any other cellular nitrogen-containing component. Formation of polymers using *L*-dopa or DHI as precursor gives place to a synthetic melanin similar to black natural eumelanin, but oxidation of pure dopamine gives place to a eumelanin-like material known as polydopamine, with extraordinary adhesion and coating properties [150]. This type of melanin is being testing for nanoencapsulation and other biotechnological applications.

The microscopic appearance of both natural and synthetic melanin is finely granular. Individual nonrefractile granules have a diameter of less than 800 nanometers that in heavily pigmented lesions can hide histological details. It is shown by low-voltage high resolution transmission electron microscopy that granules are stacking sheets of protomolecules forming an onion-like nanostructure. The intersheet spacing within these structures is between 3.7 and 4.0 Å consistent with noncovalent π-π stacking in heteroaromatic systems [151]. This stacked planar structure seems to indicate that the key photoprotective properties of skin melanin are derived from the energy absorption aromatic units in the molecular chemical structure rather than other optical properties related to the granular supramolecular organization.

7. Melanin Functions

As melanin is located in many animal tissues, and in practically all living organisms, it is not surprising that melanin has a lot of different functions. Most of these functions are related to protection against external insults and to confer environmental advantages to melanized cells.

Beginning with the anthropologic point of view, melanin has a high biomedical interest mostly due to its physiological photoprotective properties. Eumelanin shows a very broad UV-visible absorption spectrum [152], and it is able to dissipate up to 90% of the absorbed energy from the sunlight radiation such as heat. This absorbed energy and conversion of heat can contribute to thermoregulation of melanized organisms, which is especially important for cold-blooded animals. Cutaneous melanin in mammalian homeothermic species dissipates most of the energy and its contribution to thermoregulation is not very important. Anyway, some heuristic theories appear to attribute a greater energetic efficiency to melanized cells [153], decreasing the necessity of mitochondrial oxidations, and evermore a "melanin theory" proposes certain advantages to Africans because of the higher melanin content. That theory has been dissected and refuted [154].

Returning to authentic science rather than pseudoscience or highly speculative theories, it is clear that skin melanin is a very efficient photoprotective factor; melanin is a natural sunscreen that functions as a broadband radiation absorbent. Thus, melanin prevents the skin from the potentially damaging effects of UV light. Exposure of human epidermis to sunlight produces melanin, causing a moderate tan effect on the skin to increase the amount of photoprotective pigment. Many epidemiological studies have shown a lower incidence for skin cancer in individuals with high amount of melanin in the skin [13, 155]. Skin is the most common site of cancer in humans, especially in those with pale skin, and UV is the main environmental factor responsible for the formation of malignant melanoma and other skin cancers.

At least two basic aspects should be considered concerning the link between cutaneous pigmentation and effects of the sunlight radiation on the skin: the wavelength of the incident light radiation and the type of melanin found in the skin. Concerning the first point, in principle there is no doubt that UV light is more harmful that the visible range of sunlight since it has higher energy. Things are not so clear for which UV region is more dangerous, because there is more than one mechanism to initiate malignancy [156, 157]. Some reports claimed that UVA (320–380 nm) is the main cause of melanin synthesis and also melanoma appearance. This mechanism is related to reactions involving directly melanin, as the pigment absorbs the energy from the radiation and originates free radicals and other injurious species [158]. On the other hand, other reports indicate that UVB (280–320 nm) seems to initiate melanoma in a pigment-independent manner, associated with a direct damaging action of that radiation on DNA [159].

Concerning the second point, the nature of melanin found in human skin, it is clear that melanin may attenuate the effects of radiation, but absorption is accompanied

by redox reactions and electron transfer processes in the polymer. Both eumelanin and pheomelanin are affected by sunlight, and they form semiquinoid-type free radical species and then typical free radical species from water, but eumelanin is quite stable and large enough to scavenge the originated species. In contrast, pheomelanin has been shown to be rather photolabile under sunlight at physiological conditions, and it is easily involved in the high production of superoxide [160]. It cannot scavenge all the derived reactive species, and thus, pheomelanin may easily become a photosensitized agent rather than a photoprotector [161].

Given the phototoxicity and the reported capacity to increase cancer risk of pheomelanin [160, 162, 163], this point is especially relevant for pale-skin individuals. The existence of some other factors related to pheomelanin but not to UV light exposition that also increase cancer risk has recently been proposed [164]. In that way, individuals with high amount of pheomelanin in their fair skin would have a greater risk of cutaneous cancer from a double point of view, on one hand, because of the photosensitizer properties of that type of melanin after incidence of sunlight radiation and, on the other hand, due to UV-independent factors that are still under investigation but are more important in fair skin [164].

Albinism is an autosomal recessive genetical disorder characterized by the incapacity to produce melanin [165]. Taking into account the photoprotective function of cutaneous melanin, it is obvious that these patients are very sensitive to sunlight, as their skin, hair, and eyes miss the protector pigment. There are several types of oculocutaneous albinism (OCA) and an X-linked ocular albinism (OA) where the cutaneous melanin is poorly or not affected [166]. Excellent reviews about albinism have been published [167, 168] and, very recently, a new one describing the latest new forms of this disease has been published [169].

The most classical one is OCA1 in Caucasians which is due to mutations in tyrosinase, the key enzyme in animal melanogenic pathway [168, 170]. However, other ethnic groups show higher incidences of other types of albinism. For example, the most common type among people of black African is OCA2 [171]. This is due to mutations in another nonenzymatic protein called protein p, homologous to the protein is encoded by the pink-eyed dilution gene in mice [172]. Other human important types of OCA are Hermansky-Pudlak syndrome [173], most prevalent among people from Puerto Rican, and Chediak-Higashi syndrome [174]. These patients suffer from other more severe conditions than hypopigmentation and photophobia, such as bleeding diathesis secondary to platelet dysfunction and lung disease (pulmonary fibrosis) or lymphofollicular malignancy, but a more detailed description of these clinical symptoms is out of the scope of this review.

Aside the photoprotection of cutaneous melanin, many other functions should be considered. Melanin, especially eumelanin, is an insoluble, resistant, and stable biopolymer, without significant degradation, and thus it is sometimes considered as a relatively inert substance, but this is not really correct. Melanins are quite reactive, and they display a series of complex structural and physicochemical properties in addition to resistance to degradation. They present charge-transfer redox activity, and they are outstanding stable radical, free radical scavenger, chelating agent for ions, binding capacity for a variety of biomolecules, and organic agents (drugs, antibiotics, and other xenobiotics). These chemical properties make melanins beneficial pigments in many ways different from sunlight absorption [175] as they can act as

(a) redox polymers, buffering the level of other intracellular redox biomolecules inside the cell;

(b) radical scavengers, for the neutralization of ROS and other reactive oxygenated species;

(c) ion chelating agent and possibly exchanger; melanin is able to chelate metal ions through its carboxylated and phenolic hydroxyl groups, in many cases with high affinity and efficiency; thus, it may serve to sequester potentially toxic metal ions, protecting the rest of the cell;

(d) polymers with strong capacity to bind a variety of organic molecules, xenobiotics, and aromatic and lipophilic compounds;

(e) a protection shield for encapsulating and isolating structures, such as fungal spores, reinforcing cell walls and insect exocuticle;

(f) semiconductor materials with high capacitance useful for nanotechnological devices.

Concerning extracutaneous animal melanins, in the eye, melanin seems to modulate the incidence of beams of light entering the eye and the RPE (retinal pigment epithelium) attached to the retina. Melanin would absorb scattered light within the eyeball, allowing greater visual acuity [175]. The pigment at the iris and choroid also helps to protect retina from intense sunlight. In agreement with that, people with blue or green eyes are more at risk for sun-related eye problems, and albinism greatly affects visual acuity. In turn, melanin in the RPE shows antioxidant properties, protecting components of RPE such as A2E from photooxidation [176]. This ability appears to decrease in humans as they grow older. Moreover, melanization of RPE also may be implicated in the downregulation of rod outer segment phagocytosis. This phenomenon has been partially related to foveal sparing in macular degeneration [177] because of the change in the redox properties. RPE melanin has been proposed to turn from a normally antioxidant polymer into a prooxidant. Finally, as a totally new but different application of ocular melanin, iris melanin provides a rich light imaging very promising for personal identification by iris recognition [178].

The presence of melanin in the inner ear was established more than a century ago, but the exact biological function of the pigment in the labyrinth has yet to be determined. It has been proposed that high frequency or intensity acoustic waves could be buffered by melanin to regulate otocytes reception and appropriate hearing. Alternatively, melanin may also function as a biological reservoir for divalent ions and as an ion exchanger, as well as an intracellular buffering

system for calcium homeostasis [179]. Supporting that function of cochlear melanin, in humans, hypopigmentation and deafness occur together in the rare Waardenburg syndrome [180]. The absence of melanocytes in the *stria vascularis* of the inner ear results in cochlear impairment, although the mechanisms for that effect are not well understood [58].

The function of neuromelanin in the human *substantia nigra* is a very interesting and intriguing issue, as other mammals have no neuromelanin in the brain [181]. It is believed that neuromelanin is also a protective molecule in the brain. In the same way that UV radiation creates an oxidative stress in the skin, the aerobic metabolism of catecholaminergic neurons can also generate a number of *o*-quinones, such as *o*-dopaminequinone and oxygen reactive species, due to the catecholic nature of the occurring neurotransmitters [63, 83]. Exposure to traces of heavy metals, especially ferric ions [182, 183], released from neuronal tyrosine hydroxylase or mitochondrial cytochromes, is also a stress factor, as this metal ion generates redox cytotoxic reactions, as the Fenton reaction. It is clear that these threats should be mitigated, and melanin seems to be a very appropriate molecule to scavenge ROS and to chelate metal ions. In turn, neuromelanin can be formed "*in situ*" from the catecholic neurotransmitters, once they have been oxidized and are not useful as neurotransmitter anymore [59].

In Parkinson's disease, there is a decrease in neuromelanin in the *substantia nigra* as a consequence of specific dropping out of dopaminergic and noradrenergic neurons. Moreover, the loss of neuromelanin observed in Parkinson's disease is accompanied by an increase in iron levels in the brain. In agreement with those considerations, a dual role for neuromelanin in the pathogenesis of that disease has been proposed [184]. On the other hand, neuromelanin should been considered as a neuroprotective agent, but it is also a molecule which accumulates a variety of potentially damaging species and also drugs such as amphetamines [185] and MPTP [186], so that this accumulation in the brain can also become a thread for neurodegeneration. In that way, neuromelanin is as a double-edged sword and currently is an issue of active research.

Melanin fulfills other diverse but related roles in a wide range of organisms different from mammals. Birds have melanin mainly in feathers, so that the pigment can contribute to photoprotection as mammal hair, but it is obvious that function does not seem to be the main one. The first role described for avian melanin was related to provide feathers with more consistency and resistance to abrasion because of the molecular structure of the pigment [187]. Many desert-dwelling birds, for example, have black plumage as an adaptation to their abrasive habitat. Melanin has also been related to chemical resistance enlarging the long life of the feathers. The diversity in color of many avian species has also been related to honest signals, response to oxidative stress [188], sexual attraction, sexual differences between male and female [76, 189], distinctive signals in creeks, epaulets and patches, and proud predominance, as for the female peacock [190]. Some of these color variations for different ornamental functions are related to melanin but also to other pigments such as carotenoids, psittacofulvins, and reflectance phenomena [191].

In reptiles and amphibians, melanin-containing cells, melanocytes and melanophores, show a greater mobility than mammalian melanocytes, with complex mechanisms of dispersion and neurohormonal regulation adapted to the main function of melanin in these animals, thermoregulation, environmental adaptation, and camouflage. Melanin is involved in the mechanism for the absorption of sunlight energy for thermoregulation, a function that is especially important for cold-blooded animals [192].

Most lizards are brown under conditions of strong illumination and green under conditions of lower light intensities [76] and chameleons are the most versatile animals related to change their skin color in response to the environment. In turn, these animals have often peritoneal pigmentation [193, 194], and these melanins are probably involved in the scavenging of toxic metals and organic molecules mechanisms during the heterophagocytosis and autophagocytosis processes.

In a different strategy for camouflage, cephalopods have melanin kept in a sac to be ejected as mechanism of protection against physical attack. This form of melanin makes up the ink used by octopus, cuttlefish, and other cephalopods as a defense mechanism against marine predators. Due to the facility for obtaining this melanin, cuttlefish has been a historical model for structural studies of this pigment [8, 38].

Going down throughout the phylogenetic scale, insects and plants use melanin again for protective purposes, but with different features. In insects, a dark melanin-like structure is formed during sclerotization of insects to strengthen exocuticle and to defend against microbial infection and invading organisms [85, 87]. This is also used in some helminthes that have a polyphenol activity important in egg shell formation where melanization is presumably part of the shell hardening as the egg matures [104]. In plants, an allomelanin film is usually formed after minor cuts or wounds in fruits or vegetal tissue, as a result of bird or insect bites. The positive correlation between levels of polyphenol oxidase and the resistance to pathogens and herbivores is frequently observed, although a definitive convincing proof of a causal relationship still has not been fully established [95].

Related to the protection, the mechanism of action of phenol oxidases on phenols to form *o*-quinone intermediates and finally melanin is also protective as it is considered a primitive form of immunity [195, 196]. These nascent *o*-quinones derived from the phenol oxidase action act as host defenses against invading microbes. In insects, that incidental melanization requires the activation of a hemolymph prophenoloxidase to its enzymatically active form, phenol oxidase, to form melanin at wound sites and around intruding microorganisms in the hemolymph. The function of the phenol oxidase is the generation of a cross-linker component that is involved in the hardening and clotting, in a possible primitive link between phenol oxidase action and the coagulation system in higher animals [92]. Plant catechol oxidases have also been stored as inactive proenzymes and, after activation, they catalyze quinones formation and browning reactions in a similar way, but the process in vegetables has a

very significant importance in postharvest fruit and vegetable management. These reactions cause deterioration and loss of food quality [197, 198]. In this regard, catechol oxidase inhibitors to prevent browning are much more important than the catechol-melanin responsible of the browning.

In the microbial world, the question is why microorganisms make melanin. It is again clear that in the microbial world melanin should be always related to protective functions against environmental insults, but UV light is not the most harmful potential insult for lower forms of life. As melanin is able to absorb almost all types of radiations, it is a good protector for very stressful conditions, as evidenced in exposition to gamma radiation. For instance, in Chernobyl, a number of data show that black highly melanized fungal species have responded to the deathly ionizing radiation with enhanced growth. Those fungi colonize stations and are able to adapt morphologically to extreme conditions due to the eumelanin [199]. This protective function of eumelanin is similar to higher animals, as, for instance, bird population with pheomelanin-based feathers which have declined due to the exposure to the radiation as results of the poorer protective properties of the latter type of melanin [200].

Assuming that melanin confers resistance to a variety of unusual adverse environmental factors and extreme conditions, it is also protective for more usual stress conditions, such as high temperature or desiccation. Melanin and related materials, such as the bacterial and fungal humic acids, confer resistance to degradative enzymes (chitinase or cellulase) of structural polysaccharides, the other component of melanized cell walls [201]. There is no doubt that survival of fungal conidia is correlated with the occurrence of melanin in the structure. In *Streptomyces*, melanin formation is a protective response to adverse environmental conditions [202]. *Bacillus subtilis* is a spore-forming bacterium that produces a coat protein that catalyzes the synthesis of a brown melanin pigment around the endospore [203]. The melanin-based protective pigment in some bacteria and fungi is also capable of encapsulating and oxidizing chemical compounds or invading organisms. *Bacillus thuringiensis* synthesizes a melanin that protects the bacteria against pesticides [135].

In relation to this, melanins can also contribute to microbial virulence by reducing the susceptibility of melanized microbial cells to host defense mechanisms. In pathogenic bacteria and fungi, melanin is extremely important as a shield against immunological host response. In *C. neoformans*, an extensive body of evidence has established a role for melanization in virulence. Melanin-deficient mutant strains were avirulent in murine models of cryptococcal infection [104]. Melanin protects from ROS and oxidative stress, as it traps free radicals and protects microorganisms against oxidizing attacks, and ROS are part of the host mechanisms against invading microbes. Similar functions have been proposed for melanin in *Aspergillus fumigates* and other pathologic fungi. In plant infection, as rice, bean, and cucumber pathogens, fungal melanin has a different particular function as the pigment is essential for cell wall penetration in appresorial processes [204]. Melanin induces the vertical penetration of the host plant tissue, so that melanized fungi are more efficient for infection than amelanotic mutants. Melanin synthesis may be involved in hyphal invasion in plant tissue (see [104] for details).

As expressed above, in invertebrates and plants, melanin is involved in the primitive innate host immune defense system against invading pathogens. In these systems, melanin is a double-edged sword as organisms, host and pathogen, synthesize melanin with antagonist purposes; host melanin is a defense against the pathogen infection, and microbial melanin is a defense against the oxidative attack and ROS release from the host. This double edge should be taken into account in studies or melanin-related treatments to prevent plant infection.

Finally, aside biological functions, melanin behaves as a semiconductor with interesting biophysical properties proposed for new biotechnological applications. Synthetic dopamine melanin has been proposed for *in situ* formation of regular coatings [150]. Uniform flat melanin films can be obtained with dopamine or other similar precursors by spray deposition, and these thin films show a stacked planar structure with interesting electronic and optical properties [205] to be used as semiconductors in electrically qualified devices and other nanotechnological applications [206]. *Yarrowia lipolytica* is biotechnologically significant yeast that produces a dopa-melanin used for the synthesis of silver and gold nanostructures effective as paint-additives [207].

8. Biochemical Pathways for Melanin Formation

According to the variety in the structure and occurrence of melanin, its biogenesis is not a single and universal process. The study on various organisms led several biosynthetic schemes, always with a general feature in all pathways: an initial phase with the enzymatic-catalyzed oxidation of phenolic precursors to quinones followed by a final phase consisting of the mostly unregulated polymerization of quinones.

The most universal and well-known route is called the Raper-Mason pathway [29, 208, 209]. Originally, this was established as the route for eumelanogenesis; later, Prota extended the pathway with a branch from *L*-dopaquinone for pheomelanogenesis [9, 45]. This pathway was established using mealworm tyrosinase to catalyze *L*-tyrosine oxidation but this is the pathway for eumelanin formation in all types of animals. *L*-tyrosine is oxidized by an enzymatically complex mechanism of action with two different activities, tyrosine hydroxylase and dopa oxidase. Both reactions occur consecutively, so that the only trace of the intermediate *L*-dopa is released from the tyrosinase active site, according to the high affinity constant between the enzyme and this *o*-diphenol [210, 211]. This initial phase of the pathway is common for both eumelanins and pheomelanins and it transforms *L*-tyrosine into *L*-dopaquinone (Figure 5, up).

This quinone is a pivotal intermediate of animal melanogenesis [36, 212]. In the presence of *L*-cysteine or alternative thiol-containing compounds, such as the physiological-occurring glutathione [213], the reaction of the thiol groups with *L*-dopaquinone is much faster than the intramolecular cyclization at neutral pH [9, 33], so that the pathway is

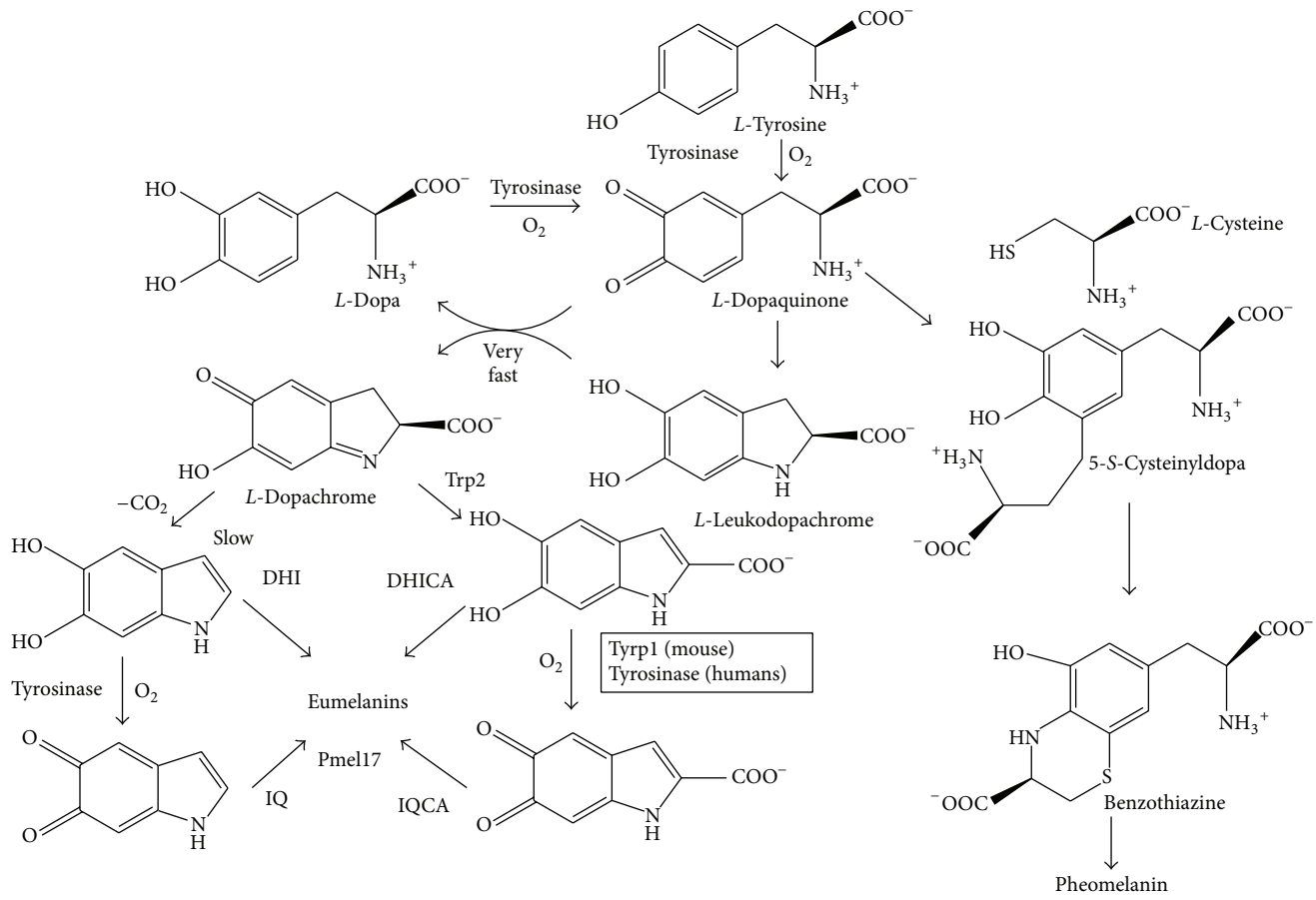

Figure 5: Raper-Mason pathway for eu- and pheomelanin formation. The initial phase until *L*-dopaquinone is common to pheomelanin and eumelanin. It consists in the oxidation of *L*-tyrosine catalyzed by the two activities of tyrosinase, tyrosine hydroxylase, and dopa oxidase. *L*-Dopaquinone is the pivotal intermediate. Addition of *L*-cysteine gives place to benzothiazine units and then to pheomelanins (right). In the absence of *L*-cysteine, eumelanogenesis takes place since *L*-dopaquinone spontaneously cycled to *L*-leukodopachrome. This indoline reacts with *L*-dopaquinone in a very fast spontaneous reaction to yield *L*-dopachrome. *L*-Dopachrome is converted to DHI and DHICA mixtures, according to Trp2 activity and decarboxylation rate. Further oxidation of these dihydroxyindoles by tyrosinase or Trp1 gives place to indolequinones; subsequent crosslink reactions between hydroxy and quinone forms lead to the polymer. In this final phase of polymerization, the Pmel17 protein located in the eumelanosome seems to have a role in the regulation and deposition of the polymer on that organelle.

directed to pheomelanin formation. The addition of *L*-cysteine can occur in different positions to yield a mixture of cys-dopa derivatives, but isomer at position 5 of *L*-dopaquinone is favored [32], predominantly 5-*S*-cys-dopa. If the conjugated thiol is glutathione, 5-*S*-glutathionyl-dopa is predominantly formed, but a peptidase converts this derivative in 5-cys-dopa by the release of glutamate and glycine residues [213]. Glutathionyl derivatives are not shown in the Figure for simplicity. The mixture of cys-dopas undergoes a series of partially known reactions [32] to benzothiazines (Figure 5, right), then to benzothiazones, and finally to pheomelanin polymer [47–49].

In the absence of *L*-cysteine or glutathione, *L*-dopaquinone undergoes a spontaneous intramolecular cyclization and it is converted into *L*-leukodopachrome to follow the eumelanin pathway. *L*-leukodopachrome, also named *L*-cyclodopa, is a strong reducing agent, so that this intermediate undergoes a spontaneous redox reaction with uncycled *L*-dopaquinone to regenerate half of the *L*-dopa and to give half *L*-dopachrome (Figure 5, left). *L*-dopachrome is the second pivotal intermediate of eumelanogenesis [214–216]. It is a relatively stable semiquinone with orange color, and it shows absorption bands at 305 and 475 nm, so that its formation rate would be widely used to determine tyrosinase activity in colorimetric assays [217]. *L*-dopachrome evolves to dihydroxyindoles with a putative decarboxylation, so that two different intermediates can be formed, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA). In fact, mixtures of both indoles are formed, depending on the several factors. In mammals, the main factor is the level of dopachrome tautomerase activity [214], also named Trp2 (tyrosinase related protein 2, EC 5.3.3.12). The ratio of DHICA/DHI units regulates the number of carboxyl groups in the final eumelanin polymer [35], and thus the size and chelating properties of the final product. In the final phase of this pathway, both DHI and DHICA are also putative substrates of tyrosinase or the other enzyme involved in the eumelanin synthesis, Trp1, to yield the corresponding

5,6-indolquinones (Figure 5) [218]. Subsequent crosslinking reactions between the reduced forms (5,6-dihydroxyindoles) and the oxidized ones (5,6-indolequinones) give place to the final eumelanin pigment. The specificity of tyrosinase and/or Trp1 to the carboxylated or decarboxylated species depends on the species. In this regard, human Trp1 seems to be different of the mouse Trp1 concerning the DHICA oxidase activity [219]. Mouse Trp1 seems to show DHICA oxidase activity [220]. Furthermore, in lower animals, a different *L*-dopachrome rearrangement enzyme seems to act, as the reaction is accompanied by decarboxylation, and DHI is obtained [87, 221].

Thus, the crucial factor for the synthesis of pheomelanin or eumelanin is the availability of free thiol compounds (cys or GSH) when *L*-dopaquinone is generated by tyrosinase. In this regard, the expression of the *Slc7a1*, a gene encoding a cystine/glutamate transporter, seems to be directly involved in the production of pheomelanin [222]. In fact, the cysteinyl compounds make a double function. On one hand, they lead the route to pheomelanin by reacting with nascent *L*-dopaquinone and, on the other hand, they regulate tyrosinase activity by direct inhibition on the tyrosinase active site [223, 224].

Extracutaneous animal melanins are formed by variants of the Raper-Mason pathway, with similar intermediates but different regulation, as tyrosinase is not expressed in all tissues. The melanosynthesis machinery in RPE [225, 226] or in cochlea [227] seems to be independent of tyrosinase and its related proteins. Concerning formation of *L*-dopa, this *o*-diphenol would be obtained from *L*-tyrosine by an alternative tyrosine hydroxylase enzyme (EC 1.14.16.2) involved in the catecholamine biosynthesis. Then, a peroxidase (EC 1.11.1.n) could be the enzyme to replace tyrosinase and generate reactive oxygenated species to provoke quinone appearance and polymerization. Melanin formation in sepiamelanin or amphibian Kupffer cells could also be formed by the initial action of peroxidase [79, 221].

The synthesis of neuromelanin in catecholaminergic neurons of the *substantia nigra* is also a variant of the Raper-Mason pathway (Figure 6). However, the initial hydroxylation of *L*-tyrosine to *L*-dopa seems to be catalyzed by neuronal tyrosine hydroxylase (EC 1.14.16.2). Immunoreactivity with tyrosinase antibodies in human pigmented neurons is negative, suggesting that tyrosinase is not involved in neuromelanin biosynthesis [228]. *L*-dopa is the product of that tyrosine hydroxylase, and the high amino acid decarboxylase (EC 4.1.1.28) activity found in these neurons converts *L*-dopa into dopamine. Dopamine can be converted into noradrenaline by the action of dopamine-β-hydroxylase (EC 1.14.17.1) in noradrenergic neurons (not shown in the figure for simplicity), and low amounts of both dopamine and noradrenaline are further oxidized by traces of reactive oxygenated species or a neuronal peroxidase to the corresponding *o*-quinones. Dopaminequinone can add *L*-cysteine or cyclize to 5,6-dihydroxyindole and then 5,6-indolequinone, and these intermediates crosslink and polymerize (Figure 6) to neuromelanin through nonenzymatic reactions [60]. Similar to Raper-Mason pathway, dopaminequinone could be transformed into dopaminechrome as intermediate to 5,6-dihydroxyindole. A dopaminechrome isomerase activity has been found in the macrophage inhibitory factor [229]. This factor is expressed in human brain and it could be involved in the rearrangements of quinone intermediates at the final phase of the neuromelanin pathway, mimicking an action similar to the Trp2 in eumelanogenesis.

In the hardening and tanning of insect cuticle (Figure 7), sclerotization and melanization, the route is initiated from *L*-tyrosine as in higher animal's melanogenesis, but *L*-dopa is decarboxylated into dopamine as in neuromelanin formation described above. However, in this particular route, the amino group of dopamine is acetylated by an *N*-acetyltransferase to *N*-acetyldopamine (NADA), the first specific precursor of the insect melanization route [230]. This intermediate is transformed into the main sclerotizing unit, 1,2-dehydro *N*-acetyldopamine, (DeNADA) [231, 232] through the formation of *N*-acetyldopamine quinone (NADAQ) and *N*-acetyldopamine quinone methide (NADAQM) throughout a series of enzymatically-catalyzed reactions as depicted in Figure 7. NADAQM undergoes an electronic aromatization rearrangement to form DeNADA. This 1,2-dehydro *N*-acetyldopamine is again an *o*-diphenol that can be again oxidized by the insect phenol oxidase to a quinonic form (DeNADAQ) to crosslink with other previous intermediates giving place to a benzodioxan derivative (not shown) and finally the dark and hard melanin material in the exocuticle of insects [233]. Similar to neuromelanin, metabolites coming from *N*-acetylnoradrenaline and units of nonacetylated DHI can be added to the final polymer [31]. This gives place to a final mixed melanin.

Although the Raper-Mason pathway and its neuronal or insect variants are the routes for the synthesis of animal melanins, the biosynthesis of allomelanins in lower organisms (taking into account allomelanins as melanins devoid of nitrogen) takes place from precursor different from *L*-tyrosine. The two main precursors of those allomelanins are diphenols, catechols, and 1,8-dihydroxynaphthalene, and these pathways usually take place in plants and fungi, respectively.

Catechol-melanins are formed by the catalytic action of plants catechol oxidases, giving quinones (Figure 8) similarly to the Raper-Mason pathway for tyrosinases, but obviously no cysteine is added to the pigment and no indolic units are either formed, in agreement with the sulfur and nitrogen economy in plants. The *o*-quinones formed by the action of catechol oxidases on catechol can react with the solvent water [234] as a redox system to yield 1,2,4-trihydroxybencene (Figure 8), although other subsequent studies propose that catechol and *o*-quinone can undergo a dismutation reaction to a semiquinone radical. This radical can dimerize to a biphenol structure or react through the oxygenated groups to yield oxygenated heterocycles [8]. The positions for crosslinking of these possible intermediates and the structure of the final polymer are undefined in spite of the early unsuccessful attempts to characterize a polymerization pattern.

DHN-melanin is formed by the pentaketide pathway. 1,8-Dihydroxynaphthalene is formed from acetyl-CoA through a carboxylative activation to malonyl-CoA which is the

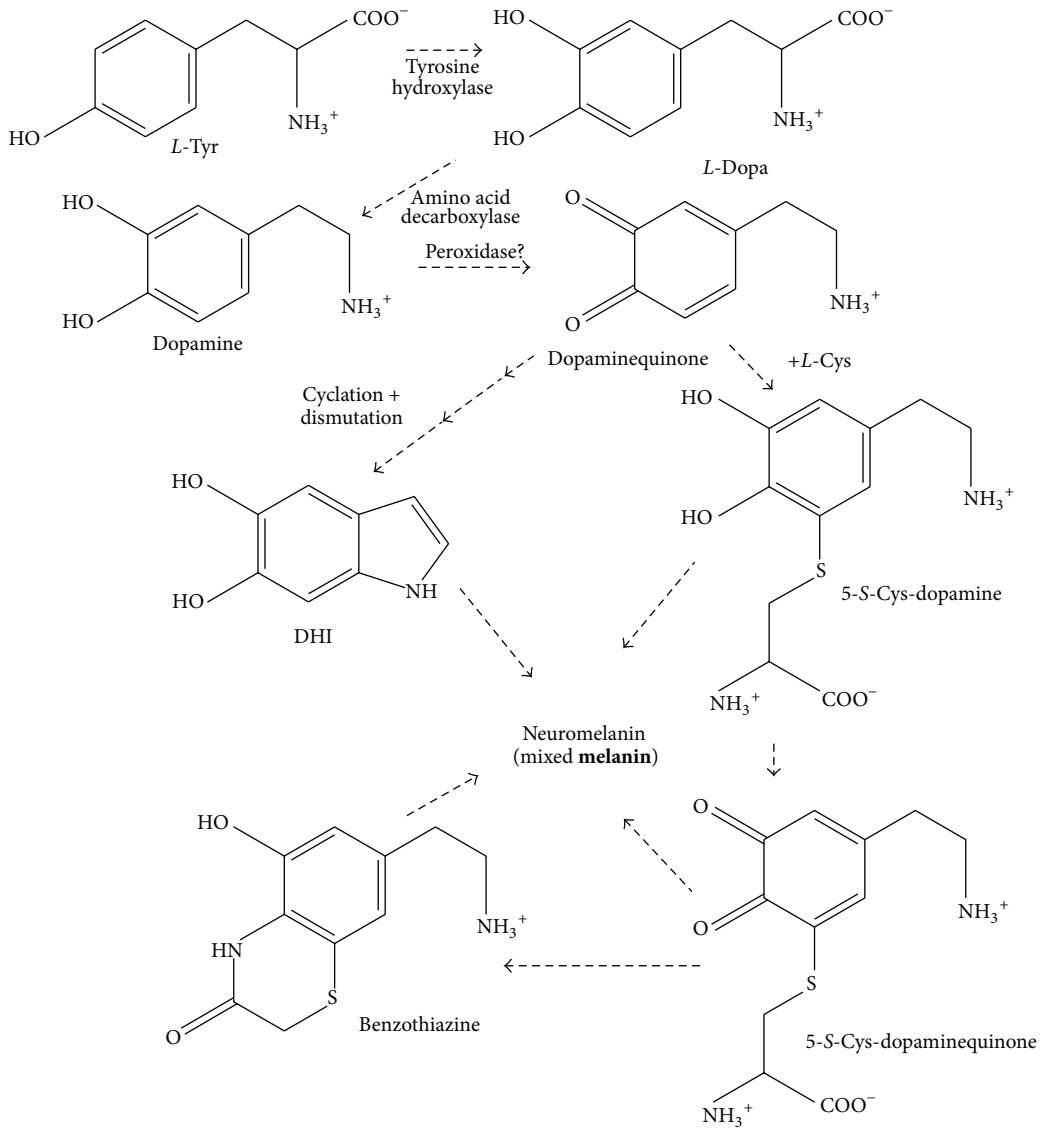

Figure 6: Neuromelanin formation. The initial precursor is *L*-tyrosine, which is hydroxylated to *L*-dopa by neuronal tyrosine hydroxylase. The high amino acid decarboxylase activity in catecholaminergic neurons yields dopamine and this is oxidized to dopaminequinone. This reaction might be catalyzed by a peroxidase or it occurs spontaneously by the action of oxygen reactive species. Similarly to *L*-dopaquinone in the Raper-Mason pathway, this quinone is pivotal in the route, giving place to 5-*S*-cysdopamine or DHI depending on the presence or the absence of *L*-cys during the dopaminequinone formation. Neuromelanin is usually a mixed melanin, as both indole and benzothiazine units are incorporated to its structure.

substrate of the pentaketide synthase system. In this route, a tetrahydroxynaphthalene is first formed [99, 100], and then that compound is transformed in 1,8-dihydroxynaphthalene (DHN) passing through scytalone and vermelone as stable intermediates (Figure 9). This dihydroxy derivative (DHN) is the characteristic substrate of fungal polyphenol oxidases, usually a laccase, to form naphthalenequinone. Similar to other melanin forming routes, those dihydroxy and quinonic forms crosslink each other in the final phase, first forming some dimers and then polymers of DHN-melanin, as shown at Figure 9 [105].

Mushroom melanin is formed from the phenolic precursor GHB by the action of a true tyrosinase (Figure 10). The initial aromatic ring is coming from chorismate, which is converted to *p*-aminophenol and conjugated with a glutamyl residue to form GHB. This monophenol is oxidized to the *o*-diphenol GDHB and subsequently *o*-quinone, GBQ. Then, glutamyl residues are mostly removed of the final pigment, as judged by the nitrogen content of the melanin [235], but the distal phase of melanization is again an undefined set of redox reactions among several intermediates. This melanin is named GHB-melanin, but sometimes also PAP-melanin as the initial substrate is *p*-aminophenol and the glutamyl moiety participates in the route but it is removed before polymerization. Mushroom tyrosinase shows greater affinity for glutaminylhydroxybenzene than for *p*-aminophenol. The

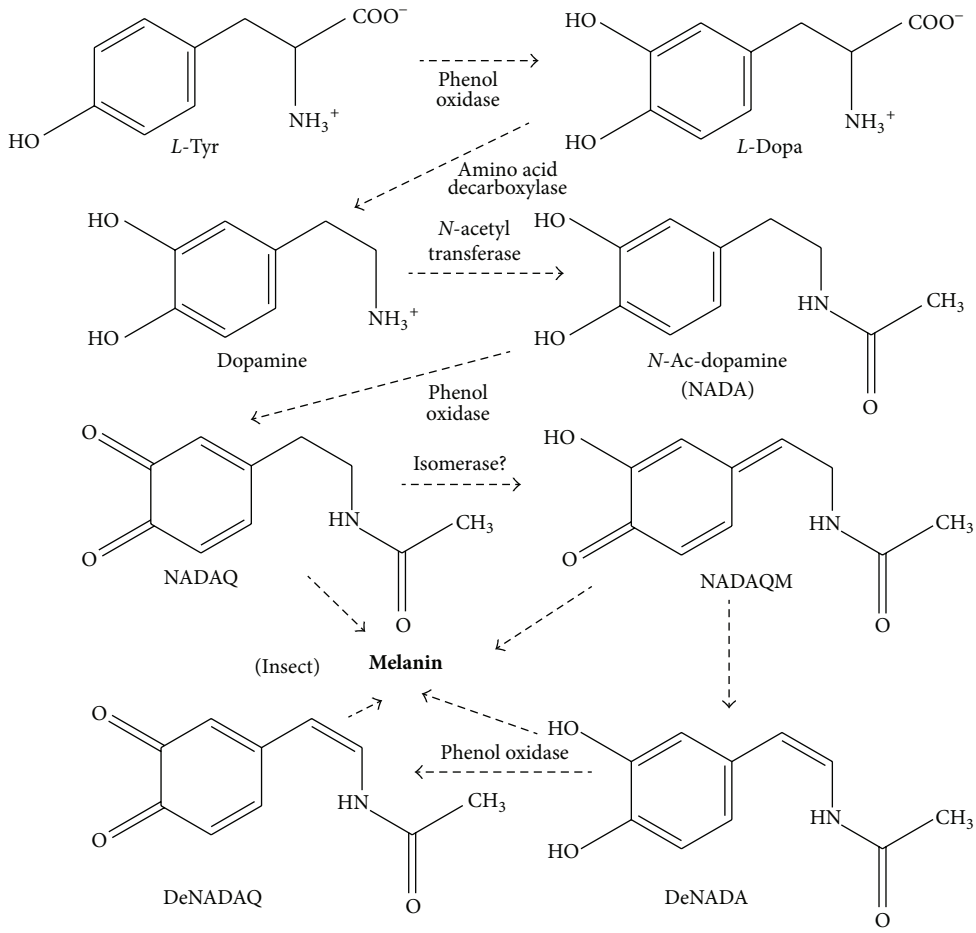

Figure 7: Insect-melanin formation: in the insect cuticle, after dopamine formation from *L*-tyrosine (see Figure 6), this intermediate is acetylated to *N*-acetyl-dopamine (NADA). This is oxidized by an insect phenyl oxidase to *N*-acetyl-dopaminequinone (NADAQ), which is tautomerized to a quinone methide (NADAQM) form. This methide is dehydrated to dehydro-*N*-acetyl-dopamine (DeNADA) and further oxidized to a new quinone (DeNADAQ) in reactions also catalyzed by phenol oxidase. The last species can dimerize to a benzodioxan and subsequently to a polymer to harden and tan the insect exocuticle.

characteristics, chemical structures, and biological properties of these typical Agaricaceae compounds are relevant because of their relevance for studies of mushroom browning during development and after harvest storage [236].

Most *pyomelanins* are formed by oxidation of homogentisate. Thus, pathway is in fact a bifurcation of the tyrosine catabolism after transamination and formation of HGA by 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27). The absence or very low homogentisate 1,2-dioxygenase (EC 1.13.11.5) activity, the enzyme catalyzing the rate-limiting step of *L*-tyrosine catabolism, allows the accumulation of homogentisate and the bifurcation of the *L*-tyr catabolic route to yield melanin as subproduct. As in other melanin forming pathways, the limiting step is the oxidation of the accumulated diphenol to the corresponding quinone, which in this particular system is a *p*-quinone (Figure 11). Subsequent redox reactions between mixed diphenols and quinones give place to the ill-defined polymer. The final steps of this polymerization pathway are also undefined and poorly studied, but, as in other systems, a phenol oxidase or laccase can be involved in the oxidation of HGA to the corresponding *p*-quinone. This form of pyomelanin is formed in some fungi, such as *Aspergillus fumigatus* [124], bacterial species, such as *Vibrio cholerae* [146], or even in human (alkaptonuria) but in bacteria similar dark yellowish pyomelanin can be formed from other *p*-dihydroxyphenols different from HGA, such as *Serratia marcescens* [141] or *Pseudomonas* [142].

9. The Phenolase System

As described in the former section, several enzymes may be involved in the different melanogenic pathways, but the most characteristic enzymatic system related to melanin formation is the phenolase system. All phenolases are mixed oxygenases, as only one atom of the atmospheric oxygen is incorporated to the phenolic substrates [237], but the system shows several forms in different organisms. Phenolases are always copper-proteins able to oxidize phenols (monophenols, *o*-diphenols, and *p*-diphenols). In general, they do not display high substrate specificity. Usually, monophenols are

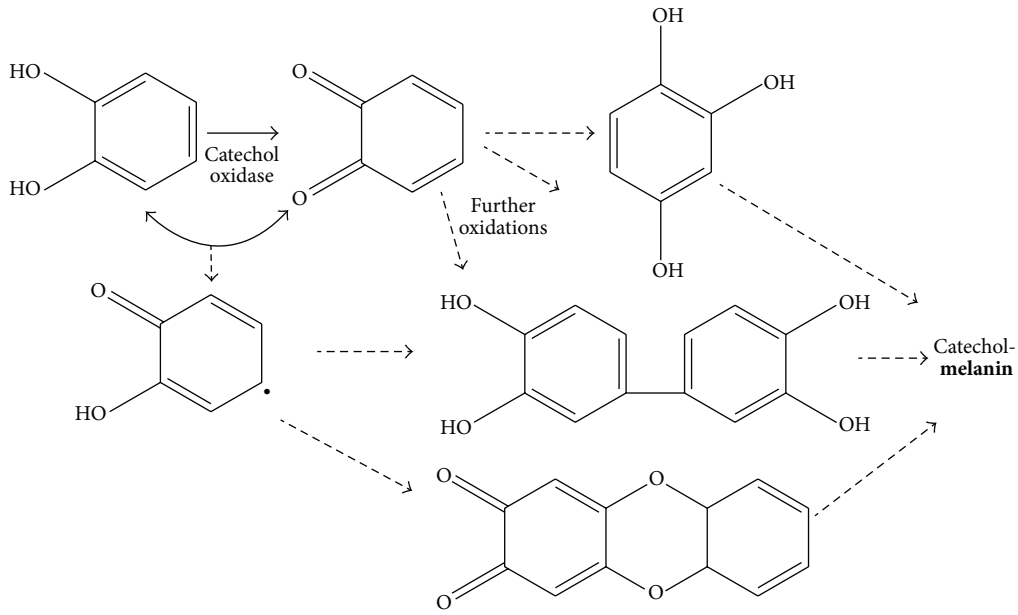

Figure 8: Catechol-melanin formation. Catechol is oxidized by a catechol oxidase, generally in plants, to yield o-benzoquinone. Dismutation reaction between catechol and o-benzoquinone can generate semiquinonic radicals (bottom). Alternatively, o-benzoquinone reacts with water to generate 1,2,4-trihydroxybenzene (right). The semiquinone radical can spontaneously react in several ways to generate aromatic biphenolic dimers or diphenylene-dioxide-2,3-quinone species and finally an undefined catechol-melanin polymer. In some species, catechol can be replaced by other catecholic precursors, such as caffeic acid, but the subsequent reactions from the corresponding o-quinone are similar to this scheme.

oxidized to *o*-diphenols, and those are further oxidized to *o*-quinones. Laccases show more affinity for *p*-diphenols, and they are involved in the synthesis of microbial melanins, DHN-melanin and pyomelanin.

Tyrosinase is the most common phenolase form in nature. It is involved in melanin biosynthesis, in animals, fungi, yeasts, and bacteria. This enzyme shows high affinity to the amino acid *L*-tyrosine, and it has two activities, tyrosine hydroxylase (monophenol hydroxylase and cresolase) and dopa oxidase (*o*-diphenol oxidase and catecholase). This is an important feature, as only the enzymes able to act on *L*-tyrosine and to display both activities are termed tyrosinase, but the phenolases displaying only the second activity and are able to act only on *L*-dopa (or other *o*-diphenol) are termed dopa (catechol) oxidases. As melanin is widely distributed in living cells, tyrosinases are found in many different organisms [238]. Actually, the first enzyme described with phenolase activity by Bourquelot and Bertrand (1895) was a tyrosinase detected in mushrooms (*Russula nigricans*), as a heat-sensitive water-soluble "ferment" that turned black the fungal tissue and *L*-tyrosine upon exposure to oxygen [113]. Concerning mammalian tissue, tyrosinase was described for the first time very early, by Gessard in 1903 [239], in an extract of horse melanoma that was able to convert *L*-tyrosine to melanin. However, Bloch [240] described the enzyme in mammalian normal skin only with dopa oxidase activity. Detection of enzymes with both activities or just with the second one puzzled the advance in the knowledge about features of this enzyme for years, but it was finally established that animal and microbial tyrosinases were able to display both activities: the hydroxylation of *L*-tyrosine and the subsequent oxidation activity of *L*-dopa [209, 241, 242].

Once it was clear the double action, the enzyme, and its mechanism of action had been deeply studied from different sources and from several points of view by different groups (i.e. [210]). Principal differences in the structure and active site of bacterial, fungal, and animal tyrosinases have also been established [212, 238]. As described above, the Raper-Mason pathway in animals is regulated not only by tyrosinase but also by two tyrosinase related proteins (TRPs) involved in the final phase of eumelanogenesis [243]. These proteins show a high homology with animal tyrosinase. Trp1 is also a Cu-dependent phenol oxidase catalyzing the oxidation of dihydroxyindoles [218], but Trp2 is a dopachrome tautomerase [214] lacking the oxidase activity due to the existence of Zn at its active site [244, 245]. There no known oxidases involved in pheomelanogenesis after *L*-dopaquinone formation.

In plants, the common form of phenolase is named catechol oxidase. The name is due to the absence of monophenol hydroxylase activity in these phenolases [92], and the preferred substrates are nitrogen-devoid *o*-diphenols, such as catechol. They are responsible of the browning of most of the fruits and leaves when they are injured and the catechols containing in the vegetal tissue are exposed to the oxygen. As browning is an undesirable process in fruit handling and after harvest marketing, the inhibition of these enzymes has important biotechnological applications.

Plants and fungi also contain laccases, that are also phenolases generally more active on *p*-diphenols than on *o*-diphenols [246]. These laccases are generally involved in

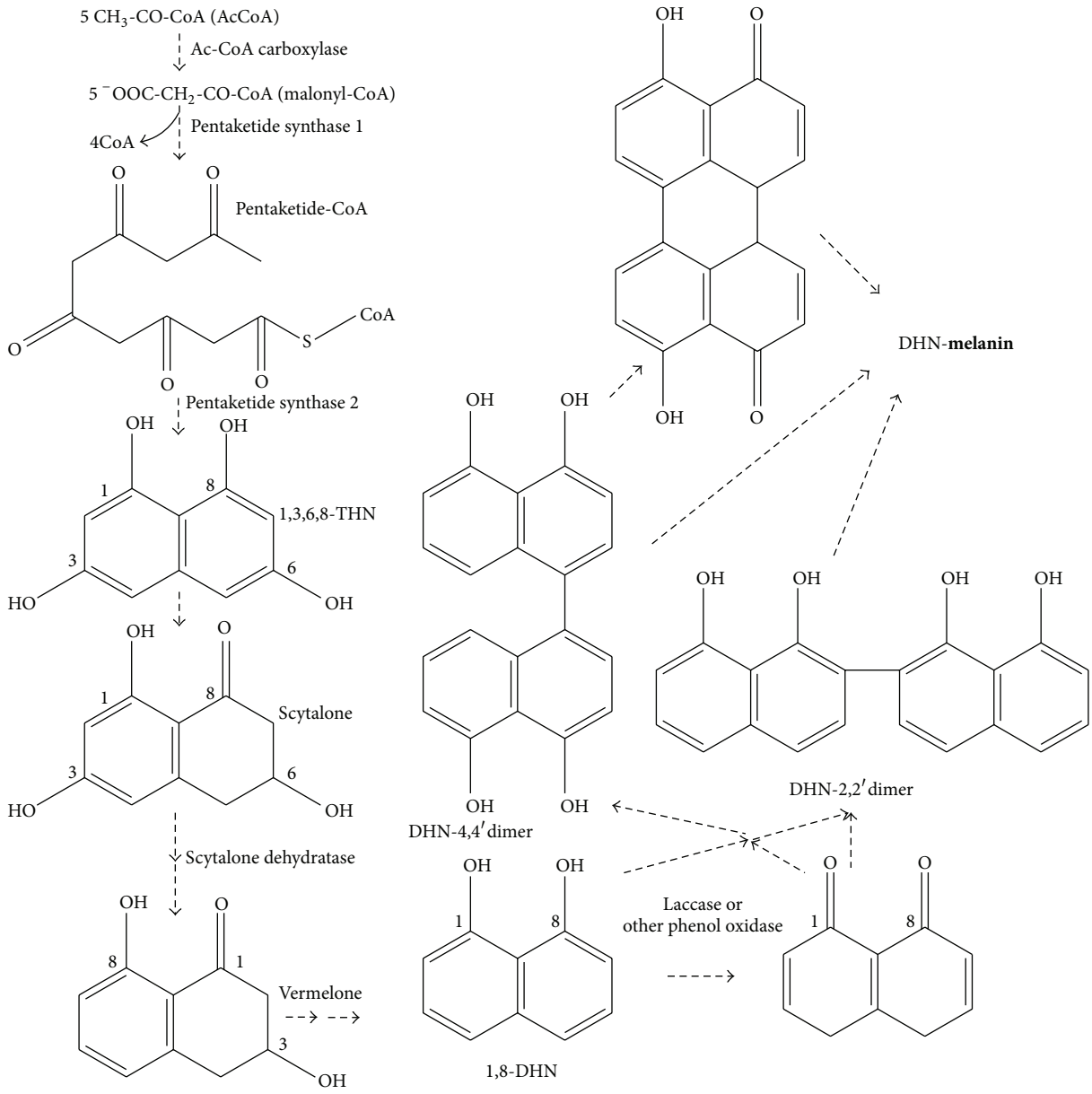

Figure 9: The pentaketide pathway (DHN-melanin formation): In this pathway, acetyl-CoA coming from intermediate metabolism is carboxylated to malonyl-CoA and five molecules give place to a pentaketide coenzyme A thioester. Subsequent cyclization by the pentaketide synthase complex yields 1,3,6,8-tetrahydroxynaphthalene. This tetrahydroxy derivative is transformed by hydration and dehydration reactions in 1,8-dighydroxynaphathalene (1,8-DHN), the main melanin precursor, passing through the formation of scytalone and vermelone as key intermediates, although other routes are possible in different fungal species. Fungal laccases and other phenol oxidases are able to catalyze their oxidation to naphthalenequinone that dimerize and polymerize to naphthalene melanin (DHN-melanin). The final polymer has undefined structure, as other types of melanin.

several processes specific of the plant or fungal species, and, only in few cases, they are just involved in melanin synthesis.

Tyrosinases, and in general phenolases, have important applications in biotechnological processes where some phenol should be oxidized. Commercially, mushroom tyrosinase is the most important source of tyrosinase due to high activity and easy extraction. Microbial sources seem to be the alternative to mushroom, as animal tyrosinases are difficult to obtain in high quantities. In this regard, bacterial laccases (EC 1.10.3.2) are still widespread in bacteria and economically attractive because purification of enzymes from bacteria is relatively simple and inexpensive, although tyrosinases occur more frequently than laccases in the bacterial kingdom [247]. Different bacterial tyrosinases [248, 249] have also been proposed for "*in vitro*" melanin preparation.

Tyrosinase from *M. mediterranea* is a plausible possibility since this species contains a multipotent phenol oxidase able to oxidize a broad range of phenols [250]. Similarly,

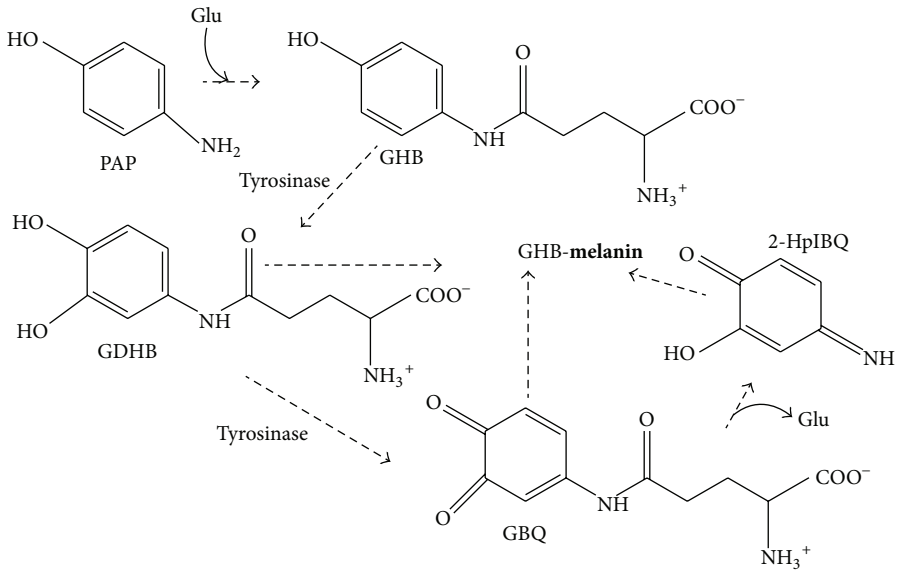

Figure 10: GHB-melanin formation: this melanin is only formed in mushroom and some other basidiomycetes. The first precursor is *p*-aminophenol which is conjugated with glutamate to form glutaminyl-hydroxy-benzene (GHB), the characteristic precursor for this route. This monophenol is oxidized by mushroom tyrosinase in a two-step reaction (see Raper-Mason pathway) to yield glutaminylbenzoquinone (GBQ). The glutamyl moiety is released to 2-hydroxy-*p*-iminobenzoquinone (HpIBQ), the real polymerizing species, although small amounts of previous intermediates carrying the glutamyl side chain can be incorporated to the final pigment during the last phase of the polymerization.

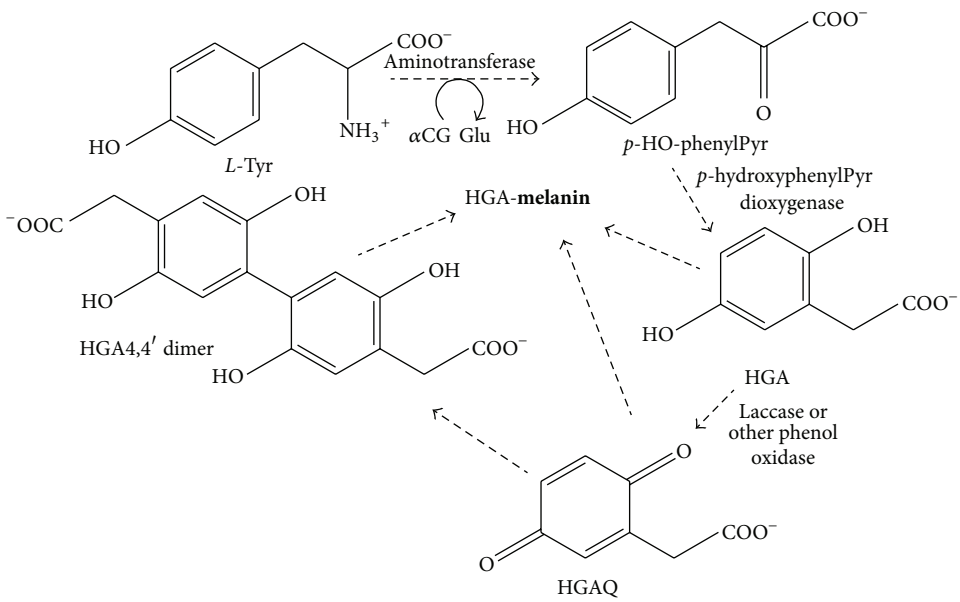

Figure 11: Pyomelanin formation pathway: this route is a bifurcation of the standard *L*-tyrosine catabolism route. This amino acid undergoes transamination to the corresponding alphaketo acid, and the action of the *p*-hydroxyphenylpyruvate dioxygenase yields homogentisate (HGA). In the absence of homogentisate dioxygenase activity, the accumulation of HGA provokes the oxidation to the *p*-quinone form (HGAQ). This oxidation of a *p*-diphenol to the *p*-quinone form can be catalyzed by laccases and other oxidase. As in other melanin formation routes, coexistence of reduced and oxidized forms spontaneously react to dimers and finally to ochre-colored polymers (HGA-melanin).

some fungal species have laccase and phenol oxidases able to form new types of melanin, as mixtures of melanin from catecholamines, dihydroxynaphthalene, and homogentisate. Finally, a few tyrosinases show a high affinity for *L*-tyrosine and low affinity for *L*-dopa, so that, in appropriate conditions to block melanin formation, they have been proposed for *L*-dopa preparation from *L*-tyrosine [251].

As stated above, inhibition of melanin synthesis is sometimes a desirable process for several purposes, mainly for cosmetical reasons in human skin, for antibrowning

treatments in fruits technology and for diminishing the virulence of some pathogenic fungi and bacteria. One of the most usual approaches for melanogenesis inhibition is the use of natural compounds as inhibitors of phenolases. Animal tyrosinases are inhibited by a number of natural and organic antioxidants used as hypopigmenting agents [252, 253]. Similarly, a great number of compounds and treatments are used as antibrowning compounds to inhibit plant catechol oxidases as enzymatic browning of fruits and vegetables is a cause of postharvest deteriorative reactions [254–256].

In the case of inhibition of microbial melanin, the target is not usually the corresponding phenolase but other enzymes related to the synthesis of melanin precursor substrate of the phenolase. The most representative inhibitors are tricyclazole for inhibition of DHN-melanin through the pentaketide pathway [100] and sulcotrione, a specific inhibitor of *p*-dihydroxyphenylpyruvate dehydrogenase [257], used for prevention of pyomelanin formation.

10. Location of Melanin Synthesis

As melanin synthesis involves quinone formation, and these reactive species are potentially cytotoxic, due to the ROS species generated during its formation and polymerization, melanin formation should be usually restricted to specific cellular and subcellular compartments. Thus, cutaneous melanin in animals is produced in melanocytes, which are specialized cells found in the basal layer between the dermis and epidermis. Hairbulbs have follicular melanocytes for transference of melanin to the hair. Typically, human skin has between 1000 and 2000 melanocytes per mm^2 that comprise around 2-3% of the total epidermal cells [258, 259]. Although all races of human beings possess a similar concentration of melanocytes in their skin, melanocytes in different ethnic groups and among some individuals differ in the level of expression of the genes related in melanin production, thereby conferring a greater or lesser melanin amounts in the skin and hair, and in the relative amounts of eumelanin and pheomelanin produced. This process is under hormonal control, mostly alpha-melanocyte stimulating hormone (MSH) which is produced from the precursor proopiomelanocortin [260].

In turn, these animal melanocytes synthesize melanin in a special organelle called melanosome [4] and move along arm-like structures dendrites of the melanocyte to be transferred to neighbor epidermal keratinocytes. In human, each melanocyte transfers melanosomes to around 30 surrounding keratinocytes, forming the melanoepidermic unit [261, 262]. The ratio of keratinocytes to melanocytes in the melanoepidermic unit changes in different skin patches and animals, and it is submitted to a genetic control, as it can be seen in beautiful nature examples, such as zebra, tiger, leopard, and giant panda [263]. Regulation of the melanocyte-keratinocyte interactions and melanin transfer is a very complex issue and an active field of research for the understanding of epidermal and follicular melanin distribution [264, 265]. Similarly, melanosomes are also formed and remain inside melanophores of reptile, amphibians, and fish. Melanin synthesis is also restricted to true melanocytes in RPE, iris, and cochlea in the eye or the inner ear, but in these systems melanosomes remain in the melanocytes and they are not transferred to surrounding cells.

There are two main types of melanosomes, spherical pheomelanosomes and ovoid eumelanosomes [266–268]. Differences in the amino acid transport systems to these suborganelles to control the availability of *L*-tyrosine and also of *L*-cysteine or glutathione inside the organelle should regulate the formation of pheo- or eumelanin.

Neuromelanin is the only type of mammalian melanins that is not formed in melanocytes, but in catecholaminergic neurons. This location is related to the double-edged sword properties sometimes attributed to neuromelanin, as melanin formation in the cytosol of neurons could have cytotoxic properties. Whereas melanin can be a dangerous route during its synthesis, once the polymer is formed, it can be a cytosolic scavenger for potentially neurotoxic subproducts, as Fe(III) or ROS due to oxidation of catecholamine neurotransmitters. It has been detected that α-synuclein could be one of the points responsible for the positive association between Parkinson disease and melanoma via its differential roles in melanin synthesis in melanoma cells and in dopaminergic neuronal cells [269, 270].

Finally, in contrast to animal melanocytes, where melanin is located almost exclusively within melanosomes, fungal and bacterial melanin can be formed in intracellular and/or extracellular spaces. In these microbial cells, prevention of intermediate quinones cytotoxicity is not so important, especially when the synthesis occurs outside the cell. Thus, in *C. neoformans,* melanin forms an electron dense layer in the cell wall external to the cell membrane [121]. This location is very similar in other fungi [99, 100], sometimes as layer and sometimes as extracellular granules at the surface of the cell wall. Melanin is also formed extracellularly in insects, plants, and many bacterial species, where the enzyme and/or the precursors are secreted to give place to melanogenesis for defensive purposes, as hardening exocuticle, cell walls, and even neutralizing host defenses in pathogenic species.

Conflict of Interests

The author declares that he does not have any conflict of interests that prevent the writing of the present paper. This paper is written as a scientific contribution on that topic, without any financial purposes.

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