STRESS-RESISTANCE OF MICROSCOPIC FUNGI FROM VARIOUS ECOLOGICAL NISHES. ROLE OF MELANIN PIGMENTS.

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Abstract. Black microscopic fungi, resistant to several stresses such as ionizing radiation, low nutrient and water content, temperature fluctuations etc., are widely distributed in various extremophilic habitats such as Arctic, Antarctic, Atakama desert, the International Space Station, places contaminated by toxic metals and ionizing radiation, and the host cells (pathogens of plants, animals and humans). Stress-resistance of these polyextremophilic fungi is provided mainly by melanin pigments, the products of polymerization of phenolic and indolic compounds. High diversity of melanin precursors as well as the biosynthesis pathways determine high variability of these pigments in different fungal species. Though formed via different precursors, polymerized melanins possess common properties. Unique melanin properties include an ability to absorb the energy of electromagnetic radiation in a wide wavelength range, permanent free radical signal, *hybrid ionic–electronic* conductance resulting in efficient electromagnetic energy dissipation, metal chelation, free *radical-scavenging*, expression of antioxidant activities. These properties determine the main functions of fungal melanins: protection against stressful environments, and its potential role in radiation energy capture and utilization in biochemical reactions. This review provides some examples of stress resistance of black microscopic fungi from various extreme ecological nishes and the properties of melanins allowing extremophilic fungi to survive in destructive to the majority of other species habitats.

Key words*: extremophilic microscopic fungi, stress tolerance, melanin pigments.*

Fungal species are responsible for many properly functioning ecosystems as well as many industrial and agricultural products. The existence of fungal community depends on its capacity to develop under different stress conditions caused by a variety of abiotic factors such as solar radiation, extremes of temperature, acidity and alkalinity, and environments of low nutritional status, low water activity, or polluted with toxic metals or xenobiotics. These physical forces act on the microbial system in such a way that impair its ability to function. The term “stress” in mycology refers to those situations that restrict or prevent the growth and reproduction of fungi [1]. Organisms that survive and thrive under conditions that are detrimental to the majority of other species have become a focus of increasing scientific attention over the last 10-20 years, with some groundbreaking discoveries of stress tolerating mechanisms**.**

Large quantities of black fungal spores have been found in early Cretaceous period deposits when many species of animals and plants died out. This time period coincides with Earth’s crossing the ‘‘magnetic zero’’ resulting in the loss of its ‘‘shield’’ against cosmic radiation [2].

Specialized fungi have been discovered not only in extreme cold, dry, salty, acidic and deep-sea habitats but also under electromagnetic radiation of different frequencies. How did this capacity evolve?

Proper adaptation to stress is critical for cell survival. Fungi possess powerful tools for environmental stress sensing, signaling and adaptation, hence, they can occupy versatile ecological niches. Fungi use different ecological strategies and have various physiological attributes, which enable them to survive and compete against other species in different nutritionally diverse niches. The outcome of such interactions and thus fungal community structure is dependent on factors such as carbon utilization patterns and fluctuating environmental factors [1]. It is therefore interesting that most extremotolerant fungi live as olygotrophs (growth under limited nutrient content in the habitat) and possess high phenotypic plasticity.Depending on the environmental conditions, these fungi can shift between yeast-like, filamentous, and meristematic , isodiametrical, resulting in a small surface/volume ratio, growth. They do not produce fertile , meiotic structures and can diverge as asexuals [1, 3].The ability to grow over a wider water-availability range than bacteria or actinomycetes also gives fungi an advantage in dominating certain ecological domains [1]. As a result extremophilic fungi exist in environments hostile to all the other living organisms.

Extremophilic fungi are grouped according to their optimal growth environments: alkalophiles show optimal growth at pH values above 10, whereas acidophiles require pH values of lower than 4 for growth; barophiles or piezophilic species require high hydrostatic pressure, endoliths live inside rocks, halophiles require high ionic strength of the growth medium (at least 0.2 M salt), and thermophiles require high temperatures, whereas psychrophiles are adapted to life at very low temperatures [1]. Combinations of the above factors are frequent and the surviving organisms are reffered to as polyextremophyles (polyextremotolerant) fungi. Stress resistance of polyextremophyles is coupled tightly to the fungus pigment apparatus [3, 4].

Pigmentation is commonly regarded as the output of secondary protective mechanisms against oxidative stress and light. Filamentous fungi secrete diverse classes of pigments as secondary metabolites, including carotenoids, melanins, flavins, phenazines and quinones. The black pigment frequently encountered in microbial systems is usually considered to be melanin (complex aggregate of quinoid pigments) or melanoprotein. Structures of melanins are characterized by the presence of multiple conjugated aromatic rings with π-electrons [5].

The term "melanin" originates from melanos – a Greek word for black. Melanin is a class of compounds found in plants, animals, fungi, and protists. The presence of various kinds of melanins in representatives of almost every large taxon suggests an evolutionary importance of melaninogenesis [1]. In general, melanins are hydrophobic pigment biopolymers formed by oxidative polymerization of phenolic or indolic compounds. Exact structures of melanins are unidentified [4]. Though formed via different precursors, polymerized melanins possess common properties. Unique melanin properties include an ability to absorb the energy of electromagnetic radiation in a wide wavelength range, paramagnetism, semiconductor properties and remarkable structural stability [4]. These properties enable melanins to provide for adaptation against extreme environments and climate changes [6]. Melanins, described in various organisms may be dark brown, black only in certain organelles, or may be totally pigmented. Dark brown or black pigments occur widely in fungi especially in polyextremophylic ones. Except for reactions to wounding, melanogenesis in fungi is restricted to certain developmental stages in special structures such as chlamydospores or microsclerotia (*Thielaviopsis*, *Verticillium*), conidia (*Aspergillus niger*), and hyaline mycelium. In the *Dematiaceae*, both hyphae and conidia are heavily pigmented (*Alternaria, Curvularia*, *Drechslera*) [7].

Fungal melanins protect against many physical and chemical environmental stressors. Fungi successfully adapted to extreme conditions demonstrate all the variety of melanins found in nature: eumelanins (black or dark brown), pheomelanins (yellow or red), and the most heterogeneous group of allomelanins, including soluble piomelanins and melanins formed from dihydroxynaphthalene compounds (DHN) [8, 9]. Melanin is produced after cessation of active growth and is therefore a likely secondary metabolite [10].

This review provides certain examples of the black microscopic fungi from various extreme ecological nishes and the properties of melanins allowing extremophilic fungi to survive in detrimental to the majority of other species habitats.

Melanin-containing extremophylic fungi from various habitats.

Melanins broadly protects fungi from an array of environmental stresses, including extreme heat and cold, drought, UV radiation, high salinity, heavy metals, and anthropogenic pollutants [11]. As a result, melanized polyextremophylic fungi are often disproportionately represented in extreme environments, such as the hot deserts of North America (Great Basin Desert), Africa (Sahara desert, Kalahari desert), Asia (Gobi desert), the Middle East (Syrian desert), Australia (Great Victoria desert) as well as the permafrost environments up to the cold, dry valleys of Antarctica, Arctic and Patagonia Antarctic [12], Atacama Desert [13], Tibetian plato [14], Deep-Subseafloor Sediments, adapted to halophylity, temperature increase and elevated hydrostatic pressure. About 200 filamentous fungi (68 %) and yeasts (32 %) were isolated from deep subsea floor sediment, [15] from inside the oceanic crust and deep sea hydrodermal vents (*Chytridiomycota, Ascomycota, and Basidiomycota*), though abundance of *Ascomycota* in the environmental samples was extremely low [16].

A wide range of rock-inhabiting fungi were isolated from limestone, marble, granite, sandstone, basalt, gneiss, dolerite and quartz, even from the most harsh environments, e.g. hot and cold deserts [17]. These stone-inhabiting microorganisms may grow on the surface (epilithic), in crevicesand fissures (chasmolithic), or may penetrate some millimetres or even centimetres into the rock pore system (endolithic), thereby gaining protection from environmental extremes and fluctuations [1].

The presence of melanin ensures the survival of microscopic fungi under the conditions of technogenic pollution. In industrial and roadside areas, an increase in the proportion of dark-colored melanin-containing fungi, which were more resistant to contamination in urban areas by heavy metals and unsaturated hydrocarbons, was observed [18, 19]. In urban conditions in air and snow samples, representatives of the genera *Cladosporium* and *Alternaria* were dominant [19, 20].

About 2000 strains representing 200 species of 90 genera have been isolated from around the Chernobyl Atomic Energy Station (ChAES) where radiation dose was from 3 to 5 orders higher than the background radioactivity [21]. Under natural conditions, extremophylic fungi often form a community with bacteria, algae, lichens, and plants [3, 22, 23]. More than 80 % of endophytic fungi that exist in the community with the herbaceous plant *Deschampsia antarctica* Desv. (Poaceae) in Antarctica produced melanin pigments [23].

Many melanized fungi belong to the *Dothideomycetes* or *Chaetothyriales* within the *Ascomycota* and free-living filamentous groups including such fungi *as Agaricus bisporus* and *Neurospora crassa* [1]. Polyextremophilic fungi also include members of the yeast, the so-called black fungi (also known as "black yeasts" or "black meristematic fungi"), a polyphyletic group of fungi which several peculiar phenotypic traits between yeast-like, filamentous, and meristematic (isodiametrical), resulting in a small surface/volume ratio growth [3, 4, 22]. A large group of melanin-containing fungi includes plant pathogens as well as pathogens of humans and animals. *Cryptoccocus neoformans* and *Aspergillus fumigatus* are the best studied. *Rhizoctonia* sp., *Sclerotinia* sp., *Verticillium* sp., are well known plant pathogens [24].

Wide melanins distribution in fungal polyextremophiles and their requirement for normal fungal development points to existence of various melanin precursors in fungi and to peculiar properties of these unique pigments.

Melanin biosynthesis in fungi.

Brief description of melanin biosynthesis in fungi demonstrating multiple precursors is presented. Detailed characterization of melanin biosynthesis in fungi can be found elsewhere [25, 26]. Fungal melanin diversity is achieved through two biosynthetic pathways: acetatemalonate pathway and shikimic acid one [19]. High molecular weight melanins are formed by oxidative polymerization of phenolic precursors. One of the most common melanin biosynthetic pathways in fungi is the polymerization of dihydroxynaphthalene (DHN pathway). This pathway has not been found in bacteria, plants, and animals. Melanins provided through this biosynthesis pathway via polymerization of precursor molecule 1,8- DHN, formed under the action of type I polyketide synthase and several oxidizing-reducing steps. The way which furnishes DHN has been termed the polyketide pathway and resides primarily in ascomycetes and related deuteromycetes [27]. A biosynthesis pathway for fungal L-dihydroxyphenylalanine (DOPA) melanin strongly resembles the pathway found in mammalian cells, though details may differ [28].

During melanin synthesis, the phenolic precursors undergo multiple oxidation and reduction steps, which can occur enzymatically or passively by spontaneous polymerizations. Melanin biosynthesis involves multiple enzymes including polyphenoloxidases (i.e. tyrosinase, laccase, catechol oxidase) - key enzymes that carry out the rate-limiting initial oxidations of phenolic melanin precursors [4, 26, 29]. These reactions are catalyzed by copper-based enzymes. Copper is important for the production of melanin by the both main pathways of melanin biosynthesis: the DHN and L-dihydroxyphenylalanine (DOPA) ones [30, 31]. Depending on the precursors, the resulting products of polymerization are the brown-black eumelanin, the yellow-red pheomelanin, or a heterogeneous group of allomelanins, including piomelanins, and a very common in fungi DHN-melanin formed via the polyketide pathway.

Eumelanins are dark brown to black pigments with 6-9 % nitrogen and 0-1 % sulfur. They are the oxidation products of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) [24]. In contrast, pheomelanins are reddish-brown pigments with 8-11 % nitrogen and 9-12 % sulfur, composed of benzothiazine monomer units [24]. Allomelanins show a heterogeneous group of pigments derived from tyrosine-metabolites of homogentisic or p-hydroxyphenylpyruvic acid (piomelanins), γ-glutamyl-4-hydroxybenzene, and catechols [24]. Melanins formed from DHN also belong to allomelanins. The last ones are very common in fungi and typically do not contain nitrogen.

Some fungal species are constitutively melanized while others melanize only under specific developmental phases (i.e. conidia, yeast filamentous growth), in response to environmental queues, or in the presence of various phenolic melanin precursors [25]. Some fungi have more than one biosynthetic pathway of melanins. For example, in *A. fumigatus*, piomelanins, synthesized from homogentisic acid (L-DOPA pathway), protect the hyphae cell wall from ROS, and gray-green DHN-melanins establish the structural integrity of the cell wall of conidia and their adhesive properties [32]. The genus *Aspergillus* comprises many species, which possess pigmented conidia of various colors. *Talaromyces marneffei* (Basionym: *Penicillium marneffei*) is capable of synthesizing DHN-melanins in conidia and DOPA-melanin in yeast cells and mycelia depending on growth conditions and supply of precursors [33]. Thus some fungi have more than one biosynthetic pathway of melanins in cells. In *Aspergillus terreus* a DHN-melanin is synthesized through a noncanonical pathway, which includes an unusual non-ribosomal peptide-synthase like enzyme and a tyrosinase . So the enzymes and mechanisms involved in the different pathways of melanin synthesis still remain obscure [34]. Thus supply of precursors and the developmental stage determine the melanin type presented in the fungal species.

There are also numerous enzymes, such as laccase, polyphenoloxidases, and perhaps peroxidases and catalases that are found in the cell walls and environs of normally nonmelanized fungi that will form black polymers from applied solutions of DOPA [4]**.** Genes of melanin synthesis in many cases assembled into clusters, thereby coordinating expression of melanin synthesizing enzymes at different stages of development of fungi [35, 36].

Structure and localization.

High diversity of melanin precursors as well as the biosynthesis pathways provide high variability of the end polymerization products. Regardless of their precursor, melanins may have similar functional groups thereby enabling comparable physicochemical properties [4]. Unfortunately, these properties are not completely understood for many fungal melanins.

Melanins are among the most stable and resistant of biochemical materials . They have been extensively studied and characterized as negatively charged amorphous compounds with quinone groups, hydrophobic and insoluble in organic solvents [28]. The polymer net structure of melanins is formed by the enzymatic and autoxidative polycondensation of various hydroaromatic compounds. The pyrrole aromatic carbons of the pigments bind covalently to the aliphatic framework via glycoside or glyceride functional groups [37]. High-resolution microscopy of fungal melanins show granular patterns, and X-ray diffraction analysis has produced patterns consistent with a stacked planar sheet structure with inter-sheet distances of approximately 0.4 nm. The mean size, mass, and unit of fungal melanin granules remain unknown [4]. A combination of SEM and TEM microscopy show these granules in various fungi. The granules are localized to the cell wall where they are likely cross-linked to polysaccharides [38].

Melanin can be found in the inner or outer layers of the cell wall depending on the fungal species [39]. Recent experimental data have shown that *C. neoformans* melanization proceeds by the attachment of melanin nanoparticles to the cell wall through chitin, chitosan, and various glucans [37]. Except for polysaccharides, melanins may additionally include other organic molecules: proteins, carohydreds and fatty acids. The presence of carbohydrates and fatty acids, covalently and noncovalently bound to melanin, was confirmed by NMR methods [37, 39], but their identification and role in melaninogenesis are still unknown [4]. There are exogenous soluble melanins, for example, piomelanins of *Ophiocordyceps sinensis* which parasitizes on insects [40]. The soluble melanins are characteristic of a number of basidiomycetes [41]. Exogenous melanins are also found in culture fluids of such extremophylic fungi such as *Cladosporium resinae* and *Aerobasidium pullulans* [42].

The synthesis of fungal melanin is similar to animal melanogenesis. It occurs inside lipids vesicles or melanosomes [43]. This is likely to protect the cell from the highly reactive free radical phenolic intermediates produced during intracellular melanogenesis [4]. On the other hand, laccase activity is associated with extracellular vesicles secreted from *C. neoformans* [44]. It provides the possibility for melanin synthesis at the fungal cell wall.

Melanized cell walls were considerably less porous than nonmelanized ones. Hence, melanin incorporation into the cell wall determines the size of the cell wall pores [45]. Possibility of melanins cross link with various macromolecules increases cell wall rigidity and cell strength [4]. On the other hand, changes in cell porosity could participate in the regulation of osmolites movement into the cell and reduce water loss. Melanization of the cell walls protects them against chemical degradation with hydrolytic enzymes [46]. In some plant pathogens, melanin accumulated in appressoria and works as a diffusion barrier. It is located between fungal plasma membrane and cell wall except for the pore where the penetration peg emerges. Appressoria accumulate glycerol; its diffusion is restrained by the pigment to create conditions necessary for host penetration [24].

Melanin properties essential for adaptation to extreme environments.

Wide distribution of black fungi in extreme environments suggests that melanins provide these organisms with some special adaptation mechanisms. Natural and synthetic eumelanin pigments share similar combinations of physico-chemical properties, such as the well assessed broadband absorption throughout the entire UV-visible range, permanent free radical signal, hybrid electronic-ionic conductor behavior, and efficient UV dissipation via excited state deactivation [4]. As melanins contain unpaired electrons, their remarkable property is that they are stable free radicals and manifest a distinctive electron spin resonance signature that is used in their identification [47]. The paramagnetic signature of melanins can be detected using electroparamagnetic spin resonance (ESR); a technique commonly used to differentiate it from other dark pigments. A comparison of the ESR spectra of melanin in various fungi showed that the g-factor of the signal was in the range 2.0036–2.0042 with a half-width of 4–7 eV. The free radical abundance in the melanin molecule depends on temperature, pH, humidity, and presence of metals [48]. In addition, this free radical population in the melanin molecule increases upon irradiation with light in a wavelength-dependent manner such that lower wavelengths are better at inducing free radicals [48]. Importantly, these free electrons generated by light absorption can further be dissipated via metabolic redox chemical reactions with other organic compounds in contact to the charged melanin [49].

Melanin pigments retain the ability to deactivate free radicals and peroxides, generated as a result of photodamage or some other stress influences. Melanized forms have high capacities for metal biosorption, with the majority of metal remaining within the wall structure [1]. The metal scavenging activity of fungal melanin allows for the bioabsorption of essential metals from rocks and other environmental niches [1].

Sequestration of iron ions has been identified as a major mechanism for the inhibitory effects of melanin on lipid peroxidation [51]. Thus these pigments exhibit profound antioxidant activity [52, 53]. In pathogenic fungi, they contribute to virulence by interfering with host defense factors, including neutralizing the oxidative burst of phagocytic cells [54]. The gene expression of melanin synthesis enzymes increases the resistance of fungi to oxidants [55, 56].

Hygroscopic character of melanin may protect cells from dessication [57], and presumably from temperature fluctuations, as melanin-containing strains of *Exophiala* [*Wangiella*] *dermatitidis* and *C.neophormans* were far more resistant to temperature shifts than their mutants lacking melanin [58]. It was revealed that ChAES *Paecillomyces lilacinum* strains with melanin content about 2-2.5 times higher than the strains from unpolluted soils were appreciably more resistant to oxidative stress [59]. Melanin-containing ChAES fungi are able to absorb not only ^{137}Cs, ^{121}Sr and ^{152}Eu, but also such radioisotopes as ^{239}Pu and ^{241}Am. This suggests that fungi could be long-term retainers of radionuclides in the environment [60]. Melanin has been shown to account for between 45 % and 60 % of ^{60}Co and ^{137}Cs incorporation into fungal hyphae [61]. Thus melanized fungi are proposed to be good candidates in bioremediation, since the organisms can potentially bind radionuclides and many other toxic substances.

Many melanized fungi are very radioresistant, requiring radiation doses exceeding 5 kGy to reduce cell survival to 10 %. Such doses are roughly 1000-fold higher than the lethal dose for humans, showing that extreme radioresistance is not limited to prokaryotes such as *Deinococcus radiodurans*, and can be achieved by eukaryotic cells [62]. Exposing polyextremophilic fungi to ionizing radiation (high-energy electromagnetic wavelengths including gamma, X-rays, and *UVA UVB* and *UVC* regions of UV spectrum zone) could bring about many deleterious effects. Due to their unique properties, melanins provide high resistance of these fungi to electromagnetic radiation. Melanin pigments absorb light in a wide spectrum range enclosing UV. Absorption intensity decreases slowly with increasing wavelengths [63].

Melanins inhibits radiation-induced toxicity in two ways. Not only does it absorb and scatter incident radiation flux, but it also effectively scavenges reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and singlet oxygen generated by electrons from water and other biomolecules (i.e. DNA, proteins) as a result of electromagnetic radiation.

Although the structure of melanins was unaffected by X-ray, γ, or UV radiation, some signal changes were detected by the ESR, indicating an increase in semiquinone radical number [4, 62]. Nontheless, melanin shielding capacity against gamma and X-rays is approximately half that of lead and twice that of carbon [62]. Fast thermal relaxation of absorbed radiation energy occurs in melanins, and the risk of dangerous photochemical reactions decreases. Thus melanins absorb light with the conversion of photons energy into heat [63, 64]. It was proposed that due to melanin's numerous aromatic oligomers containing multiple π-electron systems, a generated Compton recoil electron gradually loses energy while passing through the pigment, until its energy is sufficiently low that it can be trapped by stable free radicals present in the pigment. Controlled dissipation of high-energy recoil electrons by melanin prevents secondary ionizations and the generation of damaging free radical species [63, 65]. These properties allow melanins to be effective protectors against electromagnetic radiation. They absorb harmful UV radiation and transform the energy into harmless amounts of heat. Unfortunately, the optical properties of individual fungal melanins remain largely unexplored and could differ substantially between species [4]. Thus protective properties of fungal melanin against electromagnetic radiation resulted from a combination of physical shielding, chemical composition, and free radical quenching [62].

Melanin as an element of pathogenic mechanisms includes sequestration of host defensive proteins, redox buffering, trapping of single electrons, superoxide anion radical dismutation, an osmotic role in penetration of the (plant) cell wall by the appressorium, and protection against hydrolytic enzymes [24]. Thus, melanin has a protective role in fungi both in the host and in the environment.

Melanized fungi have been discovered in extreme cold, dry, salty, acidic climate, deep-sea habitats, zones depleted of nutrients, etc. Moreover, quite a wide taxonomic range of yeast-like and mycelial fungi have evolved potent radiation resistance [66, 67]. (25 %) fungal species from the Nevada Test Site contained melanin or other pigments [60], and up to 40 % of all fungi isolated from the Chernobyl 4th block reactor contained melanin [60]. These noticeably exceeded the ratio of melanin-containing fungi, found in environments with background radioactivity.

Melanin-containing fungi from the Chernobyl zone. Proposed energy-harvesting role of pigments.

The resistance of melanized fungi exposed to cosmic radiation on the surface Mir Space craft and International Space Station, and the presence of numerous melanized fungal species in the damaged nuclear reactor at Chernobyl suggest that melanins also play a pivotal role in protection from ionizing radiation (reviewed in [68]). The detailed description of Chernobyl fungi can be found elsewhere [69]. Our aim is to unravel how radiation energy could be transformed into biochemical one to support existence of these fungi under limited carbon resourses.

Some experimental observations showed that melanin was synthesized not only for the purposes of protection from ionizing radiation. For example, in high elevated regions inhabited by melanin-containing fungi the background radiation levels were approximately 500–1,000 higher than at sea level, which amounts to a dose of 0.50-1.0 Gy/year. Since the overwhelming majority of fungi, containing melanin or not, can withstand doses up to $1.7 \cdot 10^4$ Gy there is no apparent requirement for melanin pigments for protection against radiation in those environments [65].

Some experiments on fungi from Chernobyl alienation zone confirm these facts. Both beta and gamma radiation promote directional growth of hyphae of Chernobyl fungi towards the source of ionizing radiation (positive radiotropism). More than 60% of fungal strains isolated from the region around ChAES exhibit positive radiotropism [70].

In addition, after repeated irradiation by artificial sources (^{137}Cs, ^{123}Te,^{109}Cd,^{121}Sn), typical Chernobyl isolates (*Hormoconis resinae*, *Aspergillus versicolor, Alternaria alternate* and others) showed significant growth stimulation in the presence of ionizing radiation, called radiostimulation. Radiostimulation of conidiospore germination was demonstrated in 10 fungal isolates of six species isolated from contaminated sites [71]. Increase in growth rate under low medium glucose was demonstrated in Chernobyl isolate *P. lilacinum* with higher melanin content than the strain from background radiation level [72]. Growth increase of extremophilic fungi under radiation exposure in nutrient poor medium was subsequently confirmed for other melanin containing fungal species: *C.neoformans*, *Exophiala(Wangiella) dermatitis* and *C. sphaerospermum* [50]. It is nessesary to note, that only high radiation level (320 peak kilovoltage [kVp]) stimulated growth of *C.neoformans* melanin containing strain [73]. Thus, high radiation level is needed for the phenomena of radiotropism and enhanced growth of fungi to proceed.

Existence of melanin-containing oligotrophs in extreme ecological nishes such as Atlantic rocks or Chernobyl alienation zone points to the fact that these fungi have adapted the ability to survive or even benefit from exposure to ionizing radiation. What are the metabolic changes leading to such adaptations?

Exposure to high-energy radiation of wild-type and albino mutant *Exophiala(Wangiella) dermatitidis* have revealed downregulation of genes of general metabolic pathways (cell cycle ones and those of amino acid metabolism), and upregulation of stress response genes which is typical for fungi under stress situation [74]. Of particular interest was the observation that the wild-type (melanin-containing) but not the albino wdpks1 strain manifested ribosomal biogenesis genes' upregulation to radiation exposure, leading the authors to suggest the possibility that melanin-derived energy was being used for protein synthesis [74]. Investigation of the response of *H. resinae* to chronic radiation resulting in cumulative doses of 2 to 3 Gy elucidated that radiation was associated with enhanced synthesis of melanin and antioxidant enzymes [75]. Two-dimentional electrophoresis revealed transient alterations at the protein expression level from various fungi species (*Cryomyces antarcticus* and *Knufia perforans* - and a species of black yeasts–*Exophiala jeanselmei* - under Mars-like conditions with subsequent recovery of the metabolic activity [76]. *P. lilacinum,* an indicator of high radiation level in ChAES zone, showed elevated melanin content, improved growth rate under low glucose, and increased resistance to oxidative stress [59]. Thus infrequent data on metabolism of melanin-containing extremophilic fungi under ionizing radiation point to activation of stress-resistant mechanisms providing their survival under high irradiation.

What evidence do we have to support the hypothesis that melanins could be similar to chlorophylls and carotenoids in capacity to harvest radioactive energy and to transform it into biological one ? Many melanized fungi are very radioresistant, requiring radiation doses exceeding 5 kGy to reduce cell survival to 10 % [62]. Such doses are roughly 1000- fold higher than the lethal dose for humans. As already mentioned, three melanized fungal species *C.neoformans, W. dermatitis* and *C. sphaerospermum* constantly exposed to ionizing radiation, approximately 500 times higher than background one under nutrient limited conditions, grew significantly faster as measured by higher colony forming units (CFU), produced more biomass and showed greater incorporation of ^{14}C-acetate than nonirradiated cells or irradiated melanin defective mutants [50]. In two Chernobyl fungi under investigation *C. cladosporioides* and *H. resinae*, high radiation (2-3 Gy) caused an increase in melanin content. It resulted in its electronic properties change as measures through ESR [76]. Irradiated melanin showed an increase by four in capacity to reduce NADH relatively to nonirradiated one. Metabolic responses of melanin-producing fungi to ionizing radiation include increasing rates of electron transfer, measured as reduction of ferricyanide by the reduced nicotinamide adenine dinucleotide (NADH) [50]. Gamma radiation-induced oxidation of melanin resulted in electric current production, especially in the presence of a reducing agent [77].

These facts apparently explain the increased metabolic activity and enhanced growth of fungal hyphae under different types of radiation, found in melanin-containing fungi. Thus the possibility of participation of melanin in active electron transport in living cells leads to a hypothetical mechanism of radiation energy utilization for the increase in metabolic activity. The biological significance of this phenomenon is still unclear and some experiments yield contradicting results: gamma radiation, UV, and visible light seem to cause a reduction of ATP levels in melanized cells of the fungus *C. neoformans* [78].

Unfortunately, we haven't sufficient evidence of CO2 fixation by fungal cells with subsequent incorporation of carbon into organic molecules. It has been shown previously only for two fungal species [79, 80]. Thus the process of radiosynthesis in fungi remains an open question.

Although melanins are remarkably stable macromolecules they are still susceptible to biodegradation. Evidence exists for melanosome degradation by hydrolytic enzymes [81]. A number of fungal species have been reported to produce manganese and/or lignin peroxidases with strong melanolytic activity [57, 68]. Thus role of melanins as C-storage molecule cannot be excluded.

Fungal melanins are unique pigments playing a pivotal role in protection against stress effects and, presumably, in energy transduction and/or C storage.

CONCLUSIONS

Studies of resistance and adaptations of terrestrial black extremophiles point that melanins confer to fungi a number of useful characteristics, allowing them to adapt practically any kind of extreme environments [4, 70].

Over recent years, physicochemical studies on synthetic and non-fungal natural melanins have provided valuable insights about the properties underlying their multiple biological functions in eukaryotic systems. Their role is not merely a protection against electromagnetic radiation. In pathogenic fungi, melanization becomes a virulence factor since melanin protects microbial cells from defense mechanisms in the infected host. In turn, some melanins are formed in fungal species where sunlight radiation is not always present, for example, endolytic fungi, deep-sediment communities, etc. Then, the redox, metal chelating, or free radical scavenging properties are more important than light absorption capacity. In addition, their double role as protectants and possible energy harvesting molecules indicate that they may have played a role in the early evolution of life on Earth, when the basic background radiation was higher than today.

Melanins exhibit physicochemical and structural characteristics not replicated by any other pigment or biomolecule. However, more studies should be performed on melanin structure in fungi, biosynthesis pathways, and identifying the role melanins of different types play in fungi. Melanin biochemistry is an active field of research from biomedical and microbiological points of view, as well as ecological, agricultural and biotechnological ones.

References:

1. Drauzio E.N., Rangel R. D., Finlay J. E., Hallsworth J.H., Dadachova K., Gadd J.M. Fungal strategies for dealing with environment- and agriculture induced stresses. *Fungal Biology,* 2018, vol. 122, pp. 602-612.

2. Hulot G., Gallet Y. Do superchrons occur without any palaeomagnetic warning? *Earth Planet Sci. Lett*., 2003, vol. 210, pp. 191-20.

3. Gostincar C., Muggia L., Grube M. Polyextremotolerant black fungi: oligotrophism, adaptive potential, and a link to lichen symbioses. *Front. Microbiol*., 2012, vol.3, pp. 390-395.

4. Cordero R.J.B., Casadevall A. Function of fungal melanins beyong virulence. *Fungal Boil. Rev*., 2017, vol. 31, pp. 99-112.

5. d'Ischia M., Pezzella A., Meredith P., Sarna T. Chemical and structural diversity in eumelanins – unexplored bio-optoelectronic materials. *Angew Chem. Int.*, 2009, vol. 48, pp. 3914-3921.

6. Roulin. A. Melanin-based colour polymorphism responding to climate change. *Glob. Chang. Biol*., 2014, vol. 20, pp. 3344-3350.

7. Durán N., Teixeira M.F.S., De Conti R., Esposito E. Ecological-friendly pigments from fungi. *Crit. Rev. Food Sci. Nutr*., 2002, vol. 42, pp. 53-66.

8. Singh S., Malhotra A.G., Pandey A., Pandey K.M. Computational model for pathway reconstruction to unravel the evolutionary significance of melanin synthesis. *Bioinformation*, 2013, vol. 9, pp. 94-100.

9. Plonka P.M., Grabacka M. Melanin synthesis in microorganisms: biotechnological and medical aspects. *Acta Biochem. Polonica*, 2006, vol. 53, pp. 429-443.

10. Toledo A.V., Franco M.E., SM, Lopez S.M., Troncozo M.I., Saparrat M.C., Balatti P.A. Melanins in fungi: types, localization and putative biological roles. *Physiol. Mol. Plant Pathol*., 2017, vol. 9, pp. 2-6.

11. Treseder K.K., Lennon J.T. Fungal traits that drive ecosystem dynamics in land. *Microbiology and Molecular Biology Rev.*, 2015, vol.79, pp. 243-262.

12. Sterflinger K., Tesei D., Zakharova K., Fungi in hot and cold deserts with particular reference to microcolonial fungi. *Fung. Ecol*., 2012, vol. 5, pp.453-462.

13. Gonçalves V.N., Cantrell C.L., Werge D.L., Fereira M.C., Soares M.A., Sacob M.A. et al. Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. *Environ. Microbiol*., 2016, vol. 18, pp. 232-245.

14. Dong C., Yao Y. Isolation, characterization of melanin derived from *Ophiocordyceps sinensis*, an entomogenous fungus endemic to the Tibetan Plateau. *J. Biosci. Bioeng*., 2012, vol. 113, pp. 474-479.

15. Rédou V., Navarri M., Meslet-Cladiére L., Barbier G., Burgaud G. Species Richness and Adaptation of Marine Fungi from Deep-Subseafloor Sediments. *Appl. Env. Microbiol*., 2015, vol. 81, pp. 3571-3573.

16. Barbier G., Vandenkoornhuyse Ph. Fungal diversity in deep-sea hydrothermal ecosystems. *Appl. Environ. Microbiol*., 2009, vol. 75. pp. 6415-6421.

17. Gorbushina A.A. 2007. Life on the rocks. *Environ. Microbiol.*, 2007, vol. 9, pp. 1613-1631.

18. Kul'ko A.B., Marfenina O.E., The distribution of microscopic fungi in soils and surface air along some Moscow roads. *Microbiology,* 2001, vol. 70, pp.613-616. (In Russ.)

19. Marfenina O.E., Kul'ko A.B., Ivanova A.E., Sogonov M.V. Microscopic fungi in urban outdoor environments. *Mikol. Fitopatol.*, 2002, vol. 36, pp. 22-32.

20. Gessler N.N., Egorova A.S., Belozerskaia T.A. Melanin pigments of fungi under extreme environmental conditions. *Prikl. Biokhim Mikrobiol.*, 2014, vol. 50, pp. 125-134. (In Russ.)

21. Zhdanova N.N., Tugay T., Dighton J., Zheltonozhsky V., McDermott P. Ionizing radiation attracts soil fungi. *Mycol. Res.*, 2004, vol. 108, pp.1089-1096.

22. Gorbushina A.A., Beck A., Schulte A. Microcolonial rock inhabiting fungi and lichen photobionts: evidence for mutualistic interactions. *Mycol. Res.*, 2005, vol. 109, pp. 1288-1296.

23. Rosa L.H., Vaz A.B.M., Caligiorne R.B., Campolina S., Rosa C.A. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* (Poaceae). *Polar Biol.*, 2009, vol. 32, pp. 161-167.

24. Belozerskaya T., Gessler N.N., Averyanov A.A. Melanin pigments of fungi. Fungal metabolites. Springer Nature Switzerland AG., 2017, 1001 p.

25. Bell A., Wheeler M.H. Biosynthesis and functions of fungal melanins. *Annu. Rev. Phytopathol.*, 1986, vol. 24, pp. 411-451.

26. Eisenman H.C., Casadevall A. Synthesis and assembly of fungal melanin. *Appl. Microbiol. Biotechnol.*, 2012, vol. 93, pp. 931-940.

27. Funa N., Ohnishi Y., Fuji I., Shibuya M., Ebizuka Y., Horinouchi S. A new pathway for polyketide synthesis in microorganisms. *Nature*, 1999, vol. 400, pp. 897–899.

28. Langfelder K., Streibel M., Jahn B., Haase G., Brakhage A.A. Biosynthesis of fungal melanins and their importance for human pathogenic fungi. *Fungal Genet Biol.*, 2003, vol. 38, pp. 143-158.

29. Wheeler M.H., Bell A.A. Melanins and their importance in pathogenic fungi. *Curr. Top. Med. Mycol.*, 1988, vol. 2, pp. 338-387.

30. Cowley R.E., Tian L., Solomon E.I., Mechanism of O2 activation and substrate hydroxylation in noncoupled binuclear copper monooxygenases. *Proc. Natl. Acad. Sci*. USA., 2016, vo1. 13, pp. 12035-12040.

31. Mauch, R.M., Cunha V.O., Dias, A.L., 2013. The copper interference with the melanogenesis of *Cryptococcus neoformans*. *Rev. Inst. Med. Trop. Sao Paulo*, 2013, vol. 55, pp. 117-120.

32. Heinekamp T., Thywißen A., Macheleidt J., Keller S., Valiante V., Brakhage A.A. *Aspergillus fumigatus* melanins: interference with the host endocytosis pathway and impact on virulence. *Front Microbiol.*, 2013, vol. 3, pp. 440-447.

33. Sapmak A., Boyce K.J., Andrianopoulos A., Vanittanakom N. The pbrB gene encodes a laccase required for DHN-melanin synthesis in conidia of *Talaromyces (Penicillium*). *PLoS One*, 2015, vol.10, e0122728.

34. Geib E., Gressler M., Viediernikova I., Hillmann F., Jacobsen I.D., Nietzsche S., Hertweck C., Brock M. A non-canonical melanin biosynthesis pathway protects *Aspergillus terreus* conidia from environmental stress. *Cell Chem. Biol.,* 2016, vol. 23, pp. 587-597.

35. Fetzner R, Seither K, Wenderoth M, Herr A, Fischer R. *Alternaria alternata* transcription factor CmrA controls melanization and spore development. *Microbiology*, 2014, vol.160, pp. 1845-1854.

36. Wang Y., Hu X., Fang Yu., Anchieta A., Goldman P. H., Hernandez D., Klosterman S.J. Transcription factor VdCmr1 is required for pigment production, protection from UV irradiation, and regulates expression of melanin biosynthetic genes in *Verticillium dahlia. Microbiology,* 2018, vol. 164, pp. 685–696.

37. Chatterjee S., Prados-Rosales R., Itin B., Casadevall A., Stark R.E. Solid-state NMR reveals the carbon-based molecular architecture of *Cryptococcus neoformans* fungal eumelanins in the cell wall. *J. Biol. Chem*., 2015, vol. 290, pp. 13779–13790.

38. Casadevall A., Nakouzi A., Crippa P.R., Eisner M. Fungal melanins differ in planar stacking distances. *PLoS One*, 2012, vol. 7, e30299.

39. Eisenman H.C., Nosanchuk J.D., Webber J.B., Emerson R.J., Camesano T.A., Casadevall A. Microstructure of cell wall-associated melanin in the human pathogenic fungus *Cryptococcus neoformans*. *Biochemistry*, 2005, vol. 44, pp. 3683–3693.

40. Dong C., Yao Y. Isolation, characterization of melanin derived from *Ophiocordyceps sinensis*, entomogenous fungus endemic to the Tibetan Plateau. *J. Biosci. Bioeng*., 2012, vol. 113, pp. 474–479.

41. Hegnauer H., Nyhlen L.E., Rast D.M. Ultrastructure of native and synthetic *Agaricus bisporus* melanins – implications as to the compartmentation of melanogenesis in fungi. *Exp. Mycology,* 1985, vol. 3, pp. 221–229.

42. Gadd G.M., de Rome L. Biosorption of copper by fungal melanin. *Appl. Microbiol. Biotechnol*., 1988, vol. 29, pp. 610–617.

43. Upadhyay S., Xu X., Lowry D., Jackson J. C., Roberson R. W., Lin X. Subcellular compartmentalization and trafficking of the biosynthetic machinery for fungal melanin. *Cell Rep.*, 2016, vol. 14, vol. 2511-2518.

44. Rodrigues M.L., Nakayasu E.S., Oliveira D.L., Nimrichter L., Nosanchuk J.D., Almeida I.C., Casadevall A. Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. *Eukaryot. Cell*, 2008, vol. 7, pp. 58–67.

45. Jacobson E.S., Ikeda R. Effect of melanization upon porosity of the cryptococcal cell wall. *Med. Mycol*., 2005, vol. 43, pp. 327–333.

46. Rehnstrom A.L., Free S.J. The Isolation and Characterization of Melanin-Deficient Mutants of *Monilinia fructicola*. *Physiol. Mol. Plant Pathol*., vol. 49, pp. 321-330.

47. Enochs W.S., Nilges M.J., Swartz H.M. A standardized test for the identification and characterization of melanins using electron paramagnetic resonance (EPR) spectroscopy. *Pigment Cell Res*., 1993, vol. 6, pp. 91-99.

48. Mostert A.B., Powell B.J., Pratt F.L., Hanson G.R., Sarna T., Gentle I.R., Meredith P. Role of semiconductivity and ion transport in the electrical conduction of melanin. *Proc. Natl. Acad. Sci.* USA, 2012, vol. 109, pp. 8943-8947.

49. Khajo A., Bryan R.A., Friedman M., Burger R.M., Levitsky Y., Casadevall A., Magliozzo R.S., Dadachova E. Protection of melanized *Cryptococcus neoformans* from lethal dose gamma irradiation involves changes in melanin's chemical structure and paramagnetism. *PLoS One,* 2011., vol. 6, p. e25092.

50. Dadachova E., Bryan R.A., Huang X., Moadel T., Schweitzer A.D., Aisen P., Nosanchuk J.D., Casadevall A. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. *PLoS One,* 2007, vol. 2, p. e457.

51. Korytowski W., Hintz P., Sealy R.S., Kalyanaraman B. Mechanism of dismutation of superoxide produced during autoxidation of melanin pigments. *Biochem. Biophys. Res. Commun*., 1985, vol. 131, pp. 659-665.

52. Olennikov D.N., Tankhaeva L.M., Rokhin A.V., Agafonova S.V. Physicochemical properties and antioxidant activity of melanin fractions from *Inonotus obliquus* sclerotia. *Chem. Natural Compounds*, 2012, vol. 48, pp. 396-403.

53. Różanowska M., Sarna T., Land E., Truscott T. Free radical scavenging properties of melanin: interaction of eu- and pheomelanin models with reducing and oxidizing radicals. *Free Rad. Biol. Med*., 1999, vol. 26, pp. 518-525.

54. Nosanchuk J.D., Casadevall A. The contribution of melanin to microbial pathogenesis. *Cell Microbiol*., 2003, vol. 5, pp. 203-223.

55. Yang Y., Fan F., Zhuo R., Ma F., Gong Y., Wan X., Jiang M., Zhang X. Expression of the laccase gene from a white rot fungus in *Pichia pastoris* can enhance the resistance of this yeast to H2O2-mediated oxidative stress by stimulating the glutathione-based antioxidative system. *Appl. Environ. Microbiol*., 2012, vol. 78, pp. 5845-5854.

56. Woo P.C.Y., Tam E.W.T., Chong K.T.K., Cai J., Tung E.T.K., Ngan A.H.Y., Lau S.K.P., Yuen K-Y. High diversity of polyketide synthase genes and the melanin biosynthesis gene cluster in *Penicillium marneffei*. *FEBS J.*, 2010, vol. 277, pp. 3750-3758.

57. Jastrzebska M.M., Isotalo H., Paloheimo J., Stubb H. Electrical conductivity of synthetic DOPA-melanin polymer for different hydration states and temperatures. *J. Biomater. Sci. Polym*., 1995, vol. 7, pp. 577-586.

58. Paolo Jr., Dadachova E., Mandal P., Casadevall A.,Szaniszlo P.J., Nosanchuk J.D. Effects of disrupting the polyketide synthase gene *WdPKS1* in *Wangiella* [*Exophiala*] *dermatitidis* on melanin production and resistance to killing by antifungal compounds, enzymatic degradation, and extremes in temperature. *BMC Microbiol*., 2006, vol. 6, p. 55.

59. Egorova A.S., Gessler, N.N.,Ryasanova L.P., Kulakovskaya T.V., Belozerskaya T.A.Stress resistance mechanisms in the indicator fungi from highly radioactive Chernobyl zone sites. *Microbiology*, 2015, vol. 84, pp. 152-158. (In Russ.)

60. Dighton J., Tugay T., Zhdanova N. Fungi and ionizing radiation from radionuclides. *FEMS Microbiol. Lett.*, 2008, vol. 281, pp. 109-120.

61. Mahmoud Y.A-G. Uptake of radionuclides by some fungi. *Mycobiology*, 2004, vol. 32, pp. 110-114.

62. Dadachova E., Casadevall A. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. *Curr. Opin. Microbiol*., 2008, vol. 11, pp. 525-531.

63. Meredith P., Sarna T. The physical and chemical properties of eumelanin. *Pigment Cell Res.*, 2006, vol. 19, pp. 572-594.

64. Riesz J., Gilmore J., Meredith P. Quantitative scattering of melanin solutions. *Biophys. J.*, 2006, vol. 90, pp. 4137-4144.

65. Schweitzer A.D., Howell R.C., Jiang Z., Bryan R.A., Gerfen G., Chen C.C., Mah D., Cahill S., Casadevall A., Dadachova E. Physico-chemical evaluation of rationally designed melanins as novel nature-inspired radioprotectors. *PLoS One*, 2009, vol. 4, p. e7229.

66. Grishkan I. Ecological stress: melanization as a response in fungi to radiation. *Extremophiles Handbook.* Tokyo: Springer, 2011, 1247 p.

67. Dadachova E., Casadevall A. Melanin and resistance to ionizing radiation. *Extremophiles Handbook*. Tokyo: Springer, 2011, 1247 p.

68. Casadevall A., Cordero R.J., Bryan R., Nosanchuk J., Dadachova E. Melanin, radiation, and energy transduction in fungi. *Microbiology Spectrum*, 2017, vol. 5, pp. 1-7.

69. Zhdanova N.N., Zakharchenko V.A., Vasilevskaya A.I., Schkolni A.T., Kuchma I.D., Artishkova L.V. et al. *Mycobiota of the Ukrainian woodland. Consequences of the Chernobyl disaster*. Kiev. Naukova Dumka, 2013, 383 p. (In Russ.)

70. Zhdanova N.N., Tugay T., Dighton J., Zheltonozhsky V., McDermott P. Ionizing radiation attracts soil fungi. *Mycol. Res*., 2004, vol. 108, pp. 1089-1090.

71. Tugay T., Zhdanova N.N., Zheltonozhsky V., Sadovnikov L., Dighton J. The influence of ionizing radiation on spore germination and emergent hyphal growth response reactions of microfungi. *Mycologia*, 2006, vol. 98, pp. 521-527.

72. Aslanidi K., Ivanova A., Gessler N., Egorova A., Belozerskaya T. A Comparative investigation of adaptation to oxidative stress factors of a strain of mycelial fungus *Paecilomyces lilacinus* from Chernobyl Atomic Energy Station and strains of the same species from territories with basic level of radioactive pollution. *Radiats. Biol. Radioekol.*, 2009, vol. 49, pp. 425-431.

73. Shuryak I., Bryan R.A., Nosanchuk J.D., Dadachova E. Mathematical modeling predicts enhanced growth of X-ray irradiated pigmented fungi. *PLoS One*, 2014, vol. 9, p. e85561.

74. Robertson K.L., Mostaghim A., Cuomo C.A., Soto C.M., Lebedev N., Bailey R.F., Wang Z. Adaptation of the black yeast *Wangiella dermatitidis* to ionizing radiation: molecular and cellular mechanisms. *PLoS One*, 2012, vol. 7, p. e48674.

75. Tugay T.I., Zheltonozhskaya M.V., Sadovnikov L.V., Tugay A.V., Farfán E.B. Effects of ionizing radiation on the antioxidant system of microscopic fungi with radioadaptive properties found in the Chernobyl exclusion zone. *Health Phys*., 2011, vol. 101, pp. 375-382.

76. Zakharova K., Marzban G., de Vera J.P., Lorek A., Sterflinger K. Protein patterns of black fungi under simulated Mars-like conditions. *Sci Rep*., 2014, vol. 4, no. 5114, pp. 1-7.

77. Turick C.E., Ekechukwu A.A., Milliken C.E., Casadevall A., Dadachova E. Gamma radiation interacts with melanin to alter its oxidation-reduction potential and results in electric current production. *Bioelectrochemistry*, 2011, vol. 82, pp. 69-73.

78. Bryan R., Jiang Z., Friedman M., Dadachova E. The effects of gamma radiation, UV and visible light on ATP levels in yeast cells depend on cellular melanization. *Fungal Biol.*, 2011, vol. 115, pp. 945-949.

79. Palmer R.J.Jr., Friedmann E.I. Incorporation of inorganic carbon by Antarctic cryptoendolithic fungi. *Polarforschung*, 1988, vol. 58, pp. 189-191.

80. Moses V., Holm-Hansen O., Calvin M. Nonphotosynthetic fixation of carbon dioxide by three microorganisms. *J. Bacteriol*., 1959, vol. 77, pp. 70-78.

81. Borovansky J., Elleder M., Melanosome degradation: fact or fiction. *Pigment Cell Res*., 2003, vol. 16, pp. 280-286.