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Ionizing Radiation: how fungi cope, adapt, and exploit with the help of melanin

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SUMMARY OF RECENT ADVANCES

Life on Earth has always existed in the flux of ionizing radiation. However, fungi seem to interact with the ionizing radiation differently from other Earth's inhabitants. Recent data show that melanized fungal species like those from Chernobyl's reactor respond to ionizing radiation with enhanced growth. Fungi colonize space stations and adapt morphologically to extreme conditions. Radiation exposure causes upregulation of many key genes, and an inducible microhomology-mediated recombination pathway could be a potential mechanism of adaptive evolution in eukaryotes. The discovery of melanized organisms in high radiation environments, the space stations, Antarctic mountains, and in the reactor cooling water combined with phenomenon of 'radiotropism' raises the tantalizing possibility that melanins have functions analogous to other energy harvesting pigments such as chlorophylls.

INTRODUCTION

Life emerged on Earth at a time when there was much higher background radiation and early life forms must have considerable radiation resistance. Although current background radiation levels are much lower than on the early Earth, earthly life still exists in a field of radiation. For example, 90% of the annual radiation dose for a person living in the US comes from natural sources such as cosmic radiation and radioactive rocks (1). However, there is considerable evidence that fungi respond to radiation in a manner that may differ from other life forms. Large quantities of highly melanized fungal spores have been found in early Cretaceous period deposits when many species of animals and plants died out. This period coincides with Earth's crossing the "magnetic zero" resulting in the loss of its "shield" against cosmic radiation (2). Additionally, it has been suggested that radiation from a putative passing star called Nemesis might have contributed to extinction events (3). Fungi in general, and especially melanized ones, are highly radioresistant when subjected to high doses of ionizing radiation under experimental conditions (4–7). Understandably, such unusual abilities of eukaryotes to survive or maybe even benefit from exposure to ionizing radiation are in contrast to the general view that radiation is uniformly harmful to life. The subject of fungal cell interactions with radionuclides is of considerable interest for environmental remediation (reviewed in 8) but that phenomenon is chemical in nature and is different from interactions with ionizing radiation.

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Consequently, in this review we will focus on the recent findings on interaction of fungi with external radiation such as fungi residing in highly radioactive environments, the radiotropism of the Chernobyl-associated fungi, fungi in space, the first attempts to decipher the mechanism of radiation energy utilization by fungi as well as some insights into the genetic effects of radiation on fungi.

FUNGI INHABITING ENVIRONMENTS WITH HIGH RADIATION LEVELS

Melanized fungi inhabit some remarkably extreme environments on the planet including Arctic and Antarctic regions and high altitude terrains, with the latter habitats being characterized by the naturally occurring higher radiation levels than lower altitudes (9). The “Evolution Canyon” in Israel is a popular site for studying adaptation of organisms to their environment. It has two slopes - north facing “European” slope and south-facing “African” slope with the latter receiving 200–800% higher solar radiation than the north slope and being populated by many species of melanized fungi such *Aspergillus niger* which contain 3 times more melanin than the same species from the north-facing slope (10). Interestingly, when species of *Alternaria*, *Aspergillus*, *Humicola*, *Oidiodendron*, and *Staphylotrichum* from both slopes were subjected to high doses (up to 4,000 Gy) of ^{60}Co radiation - the isolates from the south slope grew at greater rates than the isolates from the north slope (11).

Among the environments with high radiation resulting from human activities - two examples stand out. First, melanized fungal species colonize the walls of the damaged reactor at Chernobyl where they are exposed to a high constant radiation field (12). Second, melanized fungal species are found in the so-called reactor cooling pool water. This water circulates through the nuclear reactor core for cooling purposes and is extremely radioactive. These pools comprise large amounts of fungi, cocci, Gram-positive rods, and some Gram-negative rods. Analysis of this reactor water microflora has led to the suggestion that high fluxes of radiation select for highly radioresistant types of microorganisms, which manifest increases in catalase and nuclease activities (13).

COMPARATIVE RADIOSENSITIVITY OF BACTERIA AND FUNGI

Bacterium *Deinococcus radiodurans* is considered the most radioresistant microorganism known with an LD_{10} for some strains approaching 15 kGy (14). The standard dose for food irradiation in the US is 1 kGy, which is considered sufficient to kill the bulk of the food-contaminating microorganisms since only a few strains of bacteria have LD_{10} values higher than 1 kGy (Table 1). Such bacteria are referred to as ionizing radiation resistant bacteria (IRRB) (14). However, many fungi, especially melanized ones are very radioresistant, with LD_{10} values approaching or exceeding 1 kGy (Table 1). This radioresistance of fungi is not widely appreciated and should be taken into consideration when gamma radiation is used for sterilization of food or medical supplies.

RADIOTROPISM OF CHERNOBYL-ASSOCIATED FUNGI

Zhdanova et al. reported that some of the fungi growing in the area around the site of 1986 Chernobyl nuclear accident had the ability of growing into and decomposing so called “hot particles” – pieces of graphite from destroyed reactor # 4 which are contaminated with various long-lived radionuclides (15,16). They termed this attraction of fungi to radiation “radiotropism”. In their more recent work, they excluded possible confounding effects of carbon on directional growth of fungi by exposing them to the external collimated beams of radiation from ^{32}P and ^{109}Cd radionuclides, which are beta- and gamma-emitters, respectively (17). The authors measured the “return angle” which they defined as an angle between the point of impingement of radioactivity in the culture vessel and the direction of growth of the distal portion of the emergent hyphum from each spore. A low return angle ($<90^\circ$) indicates

mean hyphal growth towards the source of radioactivity and a high angle (90–180°) – the growth away from the source. Fungi used in the experiment were either isolated from the contaminated Chernobyl zones, or isolated before the explosion or from the remote sites. Altogether 27 responses of interactions between fungal isolates and radiation source were investigated. Of these, 18 (66.7%) showed positive stimulation of growth towards the radiation source (low mean return angle), and eight showed no response. Examples of results showing the mean “return angle” are given in Fig. 1. Statistically significant directed growth to the ^{109}Cd source of radiation was seen for *Penicillium roseopurpureum* 147 (from contaminated Red Forest soil), *P. hirsutum* 3 (hot particles), *Cladosporium cladosporioides* isolates 60 and 10 (from the 4th Block reactor room). *C. sphaerospermum* 3176, although isolated from control uncontaminated soil also showed a positive response. A trend towards directional growth, though not statistically significant, was observed for *C. cladosporioides* 396 and *Paecilomyces lilacinus* 101 (both isolated from uncontaminated soils) and for *Penicillium lanosum* (from the 4th Block) and *Paecilomyces lilacinus* 1941 (Red Forest soil), both of which were originally isolated from areas of high levels of radiation. The authors concluded that both beta and gamma radiation promoted directional growth of fungi from contaminated and clean areas towards the sources of ionizing radiation.

In their later work, published in 2006–2007, the same group investigated the influence of external radiation from ^{121}Sn (low energy gamma-emitter) and ^{137}Cs (high energy mixed beta- and gamma-emitter) not only on hyphal growth of fungi from radioactively contaminated Chernobyl regions versus controls but also on their spore germination (18,19). They observed that radiation promoted spore germination in species from contaminated regions, which they called “radiostimulation”. Contrary to their previous results (17) they observed the “radiostimulation” only for the species from contaminated regions but not for isolates from the clean areas. They named this phenomenon “radioadaptive response”. They also observed the same results for responses of fungi from contaminated areas to light (20). However, though the presence of adaptive properties in fungi exposed in the long term to elevated radiation levels are very likely, the limitations of the experimental work reported in (18–20) might interfere with the authors’ ability to observe the radiostimulation for fungi from the clean areas as well. For example, the activity of the radioactive sources used in the later studies (18–20) was approximately 1,000 lower than used in earlier work (17) which might have been insufficient to promote the hyphal growth. This may have been the case especially for low energy ^{121}Sn ; also, the beta particles from ^{137}Cs might have been absorbed by the material of the Petri dish with the fungi as the collimated beam was coming from beneath and thus have not contributed to the actual radiation doses which could have been overestimated.

FUNGI INHABITING THE SPACE CRAFT

Another high radiation environment where fungi have adapted is orbiting spacecraft. Analysis of the atmosphere in the Russian orbital station Mir revealed the ubiquitous presence of many microorganisms (21). The likely sources of contamination of the space station are flight materials during manufacturing and assembly, the delivery of supplies to the space station, the supplies themselves, and secondary contamination from the crew and any other biological material on board, e.g. animals, plants and microorganisms used in scientific experiments (21). Fungal contamination poses certain threats to the well-being of the crew not only because some of those fungi are potential human pathogens but also because fungi possess powerful enzymatic systems and secrete various metabolites capable of degrading structural materials inside the spacecraft – from polymers to various alloys.

The survey of the environmental contamination on board of the International Space Station (ISS) revealed many fungal species on the surfaces and in the air (Table 2) with *Aspergillus* sp., *Penicillium* sp., and *Saccharomyces* sp. being the most dominant genera among fungi. A

diverse *Aspergillus* population was recovered (13 species), whereas diversity was less pronounced in the case of *Penicillium* (5 species) and *Cladosporium* (4 species) (22). The levels of ionizing radiation that these fungi encounter in the space stations – approximately 4 cGy per year (23) are not fungicidal (4–7,13) and allow fungi to thrive provided the humidity levels are sufficient. Interestingly, many of the microorganisms inhabiting the space station – both bacteria and fungi – were found to be pigmented or melanized, which hints at the usefulness of pigments presence in those cells under the extreme conditions.

Another important microbiology-related aspect of space flight is the possibility of spacecraft-inhabiting microorganisms changing their properties to such an extent that they become dangerous for the Earth's inhabitants when the space craft returns to Earth. Most likely such microorganisms would be located on the outside surfaces of the craft where they would be exposed to the extremes of open space. To investigate such possibility the researchers conducted “Biorisk” experiment (24). The electron-microscopy investigation of *Aspergillus versicolor* and *Penicillium expansum* exposed to open space conditions for 7 months revealed many morphological changes, which apparently allowed those fungi to survive. For example, the polysaccharide capsule and melanin layer in *P. expansum* were significantly increased in comparison with control samples, as well the numbers of mitochondria and vacuoles in space-exposed fungi were much higher than in controls.

SUGGESTED MECHANISM OF RADIATION ENERGY UTILIZATION BY FUNGI

Given the resilience and adaptability of fungi to ionizing radiation environments and that many fungi make melanin, we hypothesized that radiation could change the electronic properties of melanin, such that the pigment could function in energy transduction and that this might enhance the growth of melanized fungi. In support of this notion, ionizing irradiation changed the electron spin resonance (ESR) signal of melanin, consistent with changes in electronic structure (25). Irradiated melanin manifested a 4-fold increase in its capacity to reduce NADH relative to non-irradiated melanin. HPLC analysis of melanin from fungi grown on different substrates revealed chemical complexity, dependence of melanin composition on the growth substrate and possible influence of melanin composition on its interaction with ionizing radiation. The interaction with ionizing radiation was studied for three fungal species - *Cryptococcus neoformans* which can be grown in both melanized and non-melanized forms depending on the presence of exogenous substrate, and two intrinsically melanized species *Wangiella dermatitidis* and *Cladosporium sphaerospermum* with the latter being one of the predominant species inhabiting the destroyed reactor in Chernobyl. XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) and MTT (2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide) assays showed increased metabolic activity of irradiated melanized *C. neoformans* cells relative to irradiated non-melanized cells, consistent with the observation that exposure to ionizing radiation enhanced the electron-transfer properties of melanin. Melanized *W. dermatitidis* and *C. neoformans* cells exposed to ionizing radiation approximately 500 times higher than background grew significantly faster as indicated by higher CFUs, more dry weight biomass and 3-fold greater incorporation of ¹⁴C-acetate than non-irradiated melanized cells or irradiated albino mutants. In addition, radiation enhanced the growth of melanized *C. sphaerospermum* cells under limited nutrients conditions. The observations that melanized fungal cells manifested increased growth relative to non-melanized cells after exposure to ionizing radiation raised the intriguing possibility that melanin can function in energy capture and utilization (25).

With regards to the possibility of fungi utilizing ionizing radiation for energy, it is interesting to note older literature reporting carbon fixation by fungi under nutrients-limited conditions (26–28). Fungi were reported to use CO₂ for the synthesis of tricarboxylic acid (TCA) cycle

intermediates. The biosynthetic function of the TCA cycle necessitates a constant supply of oxaloacetate, succinyl-CoA and 2-oxoglutarate, and those reactions that replenish the supply of TCA cycle intermediates that have been termed anaplerotic. Various enzymes have been implicated in the anaplerotic fixation of CO₂ by microorganisms and most reports specify pyruvate and phosphoenolpyruvate carboxylases and phosphoenolpyruvate carboxykinase as the major activities. This CO₂ fixation takes place in white light and leads to increase in biomass as opposed to dark fixation as part gluconeogenesis, which does not lead to a net gain of carbon. It is tempting to suggest that under limited nutrients conditions melanized fungi might use this mechanism of CO₂ fixation by utilizing transduced by melanin energy of ionizing radiation instead of white light and perhaps this should be tested experimentally in future work.

Apart from a role in energy transduction, melanin appears to have significant radioprotective properties. Non-melanized *C. neoformans* and *Histoplasma capsulatum* are highly resistant to radiation but the presence of melanin further enhanced survival at higher doses. The current perception of melanin radioprotective properties is that it quenches the cytotoxic short-lived free radicals and thus prevents DNA damage. However, we also hypothesized that the radioprotective properties of melanin in microorganisms resulted from a combination of physical shielding and quenching of cytotoxic free radicals. When melanin 'ghosts' isolated from melanized cells were crushed - they lost much of their radioprotective shielding properties indicating that the spherical arrangement of melanin particles in the hollow shell contributed to radioprotection. We concluded that melanin protected fungi against ionizing radiation and its radioprotective properties were a function of its chemical composition, free radical quenching and spherical spatial arrangement (29).

GENETIC EFFECTS OF RADIATION ON FUNGI

Some insights into the genetic effects of ionizing radiation on fungi can be obtained from the studies involving *S. cerevisiae*. Kimura et al. utilized DNA microarray to investigate a post-irradiation gene expression profile in yeast cells exposed to X-rays and gamma-rays (30). Microarray analysis revealed that both X- and gamma-rays up-regulated genes related to cell cycle and DNA processing, cell rescue defense and virulence, protein and cell fate, and metabolism (Fig. 2). Likewise, for both type of rays, the down-regulated genes belonged to mostly transcription and protein synthesis, cell cycle and DNA processing, control of cellular organization, cell fate, and C-compound and carbohydrate metabolism categories. The changes for gamma-rays-irradiated cultures were observed later than for X-ray irradiated ones. The authors attributed the time-course differences to the differences in linear energy transfer between low-energy X-rays and high-energy gamma rays. Bennett et al. investigated what genes in *S. cerevisiae* are actually responsible for resistance to ionizing radiation and found that many of these genes were responsible for such important functions such as repair (RAD50, RAD51), recombination (HRP1), chromosome stability (CHL1, CTF4), endocytosis (VID21), ubiquitin degradation (GRR1), transcription (BUR2) and some others (31). A survey of *Ustilago maydis*, also known for its extreme radiation resistance revealed the similar set of genes (32). The authors concluded that the survival of *U. maydis* after exposure to high doses of radiation is a result of levels/actions of proteins involved in DNA repair rather than of the presence of specialized recombination system such as in *D. radiodurans* and that the biotrophic nature of *U. maydis* led to the emergence of an efficient DNA repair system (32). Interestingly, many of the radiation resistance genes share significant homology with human genes which might be exploited in the development of novel radiation-based cancer therapies.

Ionizing radiation generates single-strand breaks (SSBs), double-strand breaks (DSBs), base damage and DNA crosslinks. Eukaryotic cells repair DSBs by two mechanisms, with the first one being homologous recombination. The second mechanism is called illegitimate recombination, or nonhomologous end joining (NHEJ), and involves end joining in the absence

of DNA sequence homology (33). Some illegitimate recombination events are characterized by a few basepairs (bp) of homology shared at the ends of the two recombination junctions, so called microhomology-mediated recombination (MHMR) (34). Chan et al. studied MHMR in *S. cerevisiae* irradiated with 50 Gy gamma-rays (35) and showed that a DSB-induced genome-wide MHMR pathway could lead to large-scale genomic rearrangements after a single DSB end invades another genomic location. Such a phenomenon may provide benefits to evolve genetic variants that have growth advantages under genotoxic stress. They concluded that inducible MHMR pathway could be a potential mechanism of adaptive evolution in eukaryotes. These observations might explain the radioadaptive response in fungi described by Zhdanova group (18–20), but are an unlikely explanation for the enhanced growth effects of irradiated melanized organisms, which responded within hours.

MELANINS AND RADIATION IN PERSPECTIVE

Melanin pigments are found in all biological kingdoms, suggesting that these compounds are ancient molecules that emerged early in the course of evolution. Melanins are complex polymers with a variety of properties that can be made enzymatically from relatively simple precursors. A remarkable aspect of melanins is their ability to absorb all types of electromagnetic radiation (36) which endows them with the capacity for both energy transduction and shielding. The findings of melanized organisms in high radiation environments such as the damaged reactor at Chernobyl, the space station, Antarctic mountains, and reactor cooling water combined with phenomenon of ‘radiotropism’ raises the tantalizing possibility that melanins have functions analogous to other energy harvesting pigments such as chlorophylls.

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Fig. 1.

Mean return angle ($\pm$ SE) of fungi when exposed to a collimated beam of gamma radiation from a ^{109}Cd source for 24 h (fungal isolates and sources in sequence are *Penicillium roseopurpureum* 147 Red Forest, *P. lanosum* reactor room, *Paecilomyces liliacinus* 101 unpolluted soil, *P. lilacinus* 1941 Red Forest, *Penicillium hirsutum* 3 hot particles, *Cladosporium sphaerospermum* 60 reactor room, *C. cladosporioides* unpolluted soil, *C. sphaerospermum* 3176 unpolluted soil, *C. cladosporioides* 10 reactor room). Adjacent histogram bars bearing the same letter are not significantly different at $P=0.05$ (reproduced with permission from ref. 17).

Fig. 2. Graphic representation of functional categories highly induced or reduced by X- and gamma-rays. The selected categories are presented as number of genes in each category in a time-course manner. (A) X-rays and (B) gamma-rays (reproduced with permission from ref. 30).

Table 1

Comparative radiosensitivity of bacteria and fungi to external gamma-radiation

Species	LD ₁₀ , kGy	Source
<i>Thermus thermophilus</i>	0.8	14
<i>Escherichia coli</i>	0.7	14
<i>Kineococcus radiotolerans</i> ^a	2	14
<i>Rubrobacter xylanophilus</i> ^a	5.5	14
<i>Deinococcus radiodurans</i> ^a	2–15	14
<i>Penicillium lutum</i> 352	0.4	6
<i>Fusarium</i> sp. 117	0.45	6
<i>Stemphylium botryosum</i> ^b	> 5	6
<i>Alternaria tenuis</i> ^b	> 5	6
<i>Cladosporium cladosporioides</i> ^b	> 5	6
<i>Cryptococcus neoformans</i> ^b	4.3	7
<i>Histoplasma capsulatum</i> ^b	6.7	7

^a Ionizing radiation resistant bacteria (IRRB)^b Melanized fungi

Table 2

Fungal species isolated from the ISS environment and their occurrence (%) in the total number of samples (adapted from ref. 22)

Number	Species	Environment	
		Surface	Air
1	<i>Aspergillus candidus</i>	0.5	-
2	<i>Aspergillus clavatus</i>	0.5	-
3	<i>Aspergillus ficuum</i>	0.5	-
4	<i>Aspergillus flavus</i>	-	2.5
5	<i>Aspergillus janus</i>	0.5	-
6	<i>Aspergillus nidulans</i>	0.9	0.8
7	<i>Aspergillus niger</i>	2.7	-
8	<i>Aspergillus ochraceus</i>	0.5	0.8
9	<i>Aspergillus phoenicis</i>	6.5	-
10	<i>Aspergillus pulvinus</i>	0.5	-
11	<i>Aspergillus sydowi</i>	3.8	-
12	<i>Aspergillus ustus</i>	0.5	-
13	<i>Aspergillus versicolor</i>	2.3	-
14	<i>Candida sp.</i>	0.5	-
15	<i>Candida parapsylosis</i>	0.5	-
16	<i>Cladosporium sp.</i>	0.9	-
17	<i>Cladosporium cladosporioides</i>	0.5	-
18	<i>Cladosporium herbarum</i>	0.5	-
19	<i>Cladosporium tenuissimum</i>	0.5	-
20	<i>Cryptococcus albidus</i>	0.9	-
21	<i>Geotrichum sp.</i>	0.5	-
22	<i>Lipomyces sp.</i>	0.5	-
23	<i>Penicillium aurantiogriseum</i>	6	1.7
24	<i>Penicillium expansum</i>	2.3	0.8
25	<i>Penicillium grabrum</i>	0.5	-
26	<i>Penicillium italicum</i>	0.9	-
27	<i>Penicillium lividum</i>	0.5	-
28	<i>Phoma sp.</i>	0.5	-
29	<i>Rhodotorula rubra</i>	0.5	-
30	<i>Saccharomyces sp.</i>	2.8	-
31	<i>Ulocladium botrytis</i>	0.5	-