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REVIEW

Reactive Oxygen Species in Regulation of Fungal Development

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Abstract—Reactive oxygen species (ROS) are formed by fungi in the course of metabolic activity. ROS production increases in fungi due to various stress agents such as starvation, light, mechanical damage, and interactions with some other living organisms. Regulation of ROS level appears to be very important during development of the fungal organism. ROS sources in fungal cells, their sensors, and ROS signal transduction pathways are discussed in this review. Antioxidant defense systems in different classes of fungi are characterized in detail. Particular emphasis is placed on ROS functions in interactions of phytopathogenic fungi with plant cells.

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More than hundred years has passed since A. N. Bach created the peroxide theory of biological oxidation (1897). It was among the first biochemical concepts on the machinery of metabolic processes in living cells. The basis for the theory of biological oxidation was a proposition on the activation of an oxygen molecule in biological systems, and it was proved experimentally via detection of hydrogen peroxide (H_2O_2) in plant and fungal cells in Bach's works. The past century marked the discovery of new reactive oxygen species (ROS) and substantially changed our opinion on the role of these compounds in biological systems. Originally, the main attention in studies of ROS was devoted to their high reactivity, their role in pathological processes, and to problems of antioxidant defense. Experimentally detected relationships of developmental processes with the action of factors increasing intracellular ROS concentration indicated that ROS act as signaling molecules regulating physiological responses and developmental processes in fungi. Considerable recent attention is focused on molecular mechanisms of ROS signal reception and transduction and modification of gene activity in response to stress factors. These investigations are required to gain insight into the developmental processes in organisms, for elaboration of bioengineering of microbiological preparations, and for creation of effective new drugs to protect people, animals, and plants from the action of pathogenic fungi, etc.

GENERATION OF ROS BY FUNGI: PHENOMENOLOGY AND MECHANISMS

The development of fungi proceeds in immediate contact with the environment. Therefore, they are constantly subjected to physical and chemical stress factors. Among environmental factors generating ROS are ionizing radiation (α-, β-, γ-, and X-rays), UV radiation (far 200-290 nm, medium 290-320 nm, and near 320-420 nm), visible light, temperature shifts, mechanical damage, etc. All these factors are natural attributes of life. They significantly influence fungal development.

The involvement of oxygen in metabolic processes of living organisms is coupled to its activation and formation of a number of highly reactive compounds. Under intra- and extracellular factors an exited singlet state of oxygen is generated ($^1O_2$) in the cell, as well as superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), peroxide radical

Abbreviations: ADS) antioxidant defense system; Glr) glutathione reductase; GPh) hydrophobins; Gpx) glutathione peroxidase; MAPK) mitogen-activated protein kinase; NOX) NADPH-oxidase; ROS) reactive oxygen species; SOD) superoxide dismutase.

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($HO_2^{\cdot}$), peroxide ion (HO_2^-), hydrogen peroxide (H_2O_2), and nitric oxide ($NO^{\cdot}$).

As indicated at present, the main source of $O_2^{\cdot-}$ in the cell is partial reduction of oxygen, releasing a H_2O molecule, in the respiration process [1, 2]. Apart from the respiratory chain, $O_2^{\cdot-}$ appears in reactions involving xanthine oxidase, microsomal monooxygenases, lipoxygenase, cyclooxygenase, and as a result of autooxidation of thiols, flavins, quinones, catecholamines, and reduction of the xenobiotic cycle [3].

The toxicity of radicals and their role in pathological processes and aging are well known [2, 4, 5]. However, recently ever more data are accumulated on specific intracellular enzymes producing ROS to regulate proliferation, differentiation, extracellular signal transduction, ion transport, and immune response [2]. One of the routes of $O_2^{\cdot-}$ formation is O_2 reduction in a reaction with NADPH catalyzed by plasma membrane oxidases. NADPH-oxidase (NOX) is a conjugated enzyme complex involving membrane-bound and cytosolic compounds [6]. It is significant that small GTPases Ras and Rac enter into the composition of NOX. These are small monomer cytoplasmic proteins. Animal Ras-homologs transfer ROS signals connected with pathological processes, particularly with tumor formation [7].

A well-known function of NOX is generation of $O_2^{\cdot-}$ as a respiratory burst component to defend cells from invasion [6]. NOX are present in all multicellular organisms, though specific functions of the majority of these enzymes have not been identified. Participation of certain NOX in microorganism development was established [6, 8]. Genes of specific animal NOX regulatory subunits were not determined in the *Dictyostelium discoideum* genome as well as genomes of plants and fungi [9].

NOX were not found in yeast *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* as well as in dimorphic fungi *Ustilago maydis* and *Cryptococcus neoformans*. *Aspergillus nidulans* and *A. fumigatus* contain a single *nox* gene, *Podospora anserina*, *Neurospora crassa*, *Coprinus cinereus*, and *Phanerochaete chrisosporium* have two *nox* genes, and three *nox* genes were found in *Magnaporthe grisea* and *Fusarium graminearum* [10].

The presence of *nox* genes is common to all the fungi forming fruit bodies, apparently linked to the participation of these genes in sexual reproduction [10]. Inactivation of *A. nidulans noxA* gene dramatically decreased ROS generation and inhibited formation of cleistothecia at early stages of development, stimulated mycelium growth, and suppressed asexual reproduction. The *noxA* derepression was coupled to development of cleistothecia and their premature maturation [9]. Silencing of *nox1* and *nox2* genes in *P. anserina* and *N. crassa* inhibited sexual development and ascospore germination [10, 11]. NOX enzymes presumably participate in ROS generation and in other stages of development. $O_2^{\cdot-}$ is generated in *D. discoideum* early in development of the multicellular organism [12]. The implication of NOX is supported by the fact that mitochondria are not of first importance in this developmental stage, as well as by detection of NADPH-oxidize activity. Thus, appearance of NADPH-oxidize activity and $O_2^{\cdot-}$ generation are related to specific stages of development in fungi.

Information on NOX regulation of symbiosis of the fungus *Epistle fistulae* and the plant *Folium perenne* is of great interest [13]. *noxA* mutants have no ability to support symbiosis and lead to the plant death, hence a main role in establishment of symbiosis is played by ROS produced by the fungus.

Another way of generation of free radicals participating in regulation of development is $NO^{\cdot}$ formation. The source of $NO^{\cdot}$ in the cell is a multidomain enzyme—nitric oxide synthase (NO-synthase). Various molecular forms of the enzyme function in different tissues of the organism [14]. It attaches several cofactors—NADPH, FAD, FMN, heme, calmodulin, and tetrahydrobiopterin, which determine its catalytic properties. The enzyme converts L-arginine into citrulline together with formation of the signaling molecule, $NO^{\cdot}$. Regulation of tonicity of blood vessels is the best-known $NO^{\cdot}$ function [14]. It turned out that the development of *Flammulina velutipes* is connected with this second messenger. Increase in NO-synthase activity comes before fruit body formation; inhibitors of the enzyme (aminoguanidine and others) suppress their formation. Fruit body formation was stimulated upon treatment of the fungus with sodium nitroprusside, increasing intracellular $NO^{\cdot}$ concentration [15]. $NO^{\cdot}$ apparently regulates sporangiophore development in *Phycomyces blakesleeanus* [16]. In *Physarum polycephalum* a gene homologous to animal NO-synthase was found. The expression of this gene increased in the process of starvation, preceding sporangium differentiation in this organism [17].

Thus, intracellular enzyme systems produce ROS required for differentiation in fungi. Increase in intracellular ROS concentration prior to the next differentiation stage leading to formation of a new cell type has been shown in different classes of molds and fungi (*D. discoideum*, *N. crassa*, *S. rolfsii*, *F. velutipes*, etc.) [12, 15, 18-20].

ROS GENERATION UPON INTERACTION OF PHYTOPATHOGENIC FUNGI WITH PLANTS

In all cases when new products appear in a system composed of different tightly interacting organisms, the question arises to which of the partners they belong. In particular, this question refers to host–parasite systems. Oxidative burst—an increase in ROS production upon interaction of phytopathogenic fungi with plants—is well known. It is accepted with rare exceptions that the unique ROS source is the plant. No consideration is given to the

potential contribution of the fungus. An illustrative example is stimulation of $O_2^{\cdot-}$ generation by potato leaves upon application of diffusate of germinated spores of *Phytophthora infestans* described in one of the classical works of Doke and coworkers [21]. However, whether there was stimulation or the ROS producer was the diffusate alone was not monitored. It was reported recently that H_2O_2 was produced at the interface between mycelium cells and the plant in tomato leaves with the penetrated fungus *Botrytis cinerea*. The authors proposed without evidence that the H_2O_2 source was the plant [22, 23].

Clear evidence of fungal contribution to ROS production common with the plant has been obtained with fungi showing such activity outside the plant. For example, spores of *M. grisea*, the activator of rice blast disease, germinating in water generated H_2O_2, $O_2^{\cdot-}$, and $OH^{\cdot}$ extracellularly [24, 25]. On the surface of healthy leaves $O_2^{\cdot-}$ and H_2O_2 production was extremely low, but it was drastically enhanced a day after the rice blast disease infection, as in the majority of similar experiments on this or other pathological systems. It is significant that spores germinating separately from the plant produced ROS with equal or even higher intensity than the spore-infected leaves [25]. This leads to the assumption that intensive ROS production on rice blast disease in infection droplets is mainly due to the fungus rather than the plant, at least in the earliest stages of disease.

An important point is that ROS are found not only on the surface of leaves but also in relevant acellular diffusates. H_2O_2 released from the cell can easily be conserved in diffusates for tens of minutes. Nevertheless, the $O_2^{\cdot-}$ lifetime is much shorter, and its presence in the diffusate may imply that cells release precursors of $O_2^{\cdot-}$ generated thereafter in the acellular medium [25].

Upon contact with fungicides, intracellular ROS formation blocking electron transport in pathogenic fungi is increased. SSF126 fungicide, for example, used against rice blast disease, stimulated $O_2^{\cdot-}$ generation by the fungus by inhibiting bc_1 complex of the mitochondrial electron transport chain [26]. An increase in peroxidation in fungal cells is one of the consequences of oxygen activation by fungicides [27-29]. In much the same way resistant plants can influence the fungus as they respond to the infection by overproduction of not only ROS but also $NO^{\cdot}$, capable of inhibiting cytochrome oxidase in the pathogenic fungus.

Peroxidase is apparently involved in $O_2^{\cdot-}$ generation by *M. grisea* spores; in any event, cyanide inhibition of spore $O_2^{\cdot-}$ generation suggests this [25].

Glucose oxidase is a flavin enzyme not typical of plants but usually present in fungi. It oxidizes glucose to gluconic acid leading to H_2O_2 formation. Glucose oxidase can be excreted to the extracellular medium, for example, as described for the soil fungus *Talaromyces flavus* [30, 31].

Glyoxal oxidase encoded by the gene *glo1* was detected in the phytopathogenic fungus *U. maydis*. This gene product is homologous to the protein of a wood destroying fungus *P. chrysosporium*. These enzyme homologs were identified in man, plants, and their fungal parasites but they were not found in yeast and other mammals. Glo1 protein is associated with plasma membrane, oxidizes small aldehydes ($C < 4$), and generates H_2O_2. This is taken to be significant for filamentous growth and parasitism (manifestation) in *U. maydis* [32].

Oxidative burst induced by elicitors, biological inducers of plant defense reactions, is a peculiar example of interaction of phytopathogenic fungus and plants with the participation of ROS. Many metabolites of phytopathogenic microorganisms, fungi in particular, possess this feature. Elicitors show various chemical origin, but the results of their action on plants are similar. Elicitors do not produce reactive oxygen themselves, but in their presence fungal cells are subject to oxidative stress from the plant. Oxidative burst produced by such elicitors as proteins elicitins [33], arachidonic acid [34], and carbon compounds of the fungal cell wall [35] has been described many times. This may be an important element in induction of other defense reactions, e.g., for phytoalexin synthesis [34].

The possibility of the reverse sequence of events, namely, induction of elicitor activity upon ROS action has been shown not long ago. It was established that upon the action of low concentrations (10-50 μM) of H_2O_2 on spores of rice blast disease activator, excretion of metabolites was induced, which, in turn, upon action on rice callus stimulated excretion of fungitoxic compounds, and, later on, callus necrosis. Both these reactions appear upon infection of rice leaves and callus by avirulent fungal stocks [36]. They are presumably induced by elicitors. It is known that some of the elicitors are linked to the fungal cell wall, while the corresponding receptors are located on the plant plasma membrane. Their contact can arise only after fungal penetration through the cell wall of the host. This requires some time. At the same time, excretion of soluble elicitors from spores provoked by the plant apparently favors an earlier triggering of defense reactions. Thus elicitor release, produced by the plant under the action of ROS, may be a manifestation of plant resistance.

Some fungal specific methods of oxygen activation are associated with toxins for which active oxygen serves as a messenger in intoxication of the host. Some toxins are direct sources of ROS; others promote ROS formation by plants. Cercosporin of fungal genus *Cercospora* is a typical example of the first group of toxins. It is a photosensitizer damaging the cells via producing singlet oxygen and $O_2^{\cdot-}$ according to a photodynamic mechanism [37]. The pure toxin produced modifications similar to infection droplets. Alterotoxins of the fungi *Alternaria* are also defined as photosensitizers, as well as dothistromin of the fungus *Dothistroma pini*, affecting pines [38]. Dothistromin generates $O_2^{\cdot-}$ and H_2O_2 upon illumination

[39]. Photoactive phytotoxin of *P. oryzae* is well known [40].

Another type of fungal toxins generates ROS in redox reactions with plant substrates in the dark. These are, in particular, naphthazarine toxins of fungal genus *Fusarium* transferring electrons from the host mitochondrial transport chain to oxygen [41]. Dothistromin acts in a similar way by redox transformations of the quinoid part of its molecule. Some of the naphthazarine toxins of *Fusarium solani* are reduced by diaphorases with subsequent formation of $O_2^{\cdot-}$, H_2O_2, and $OH^{\cdot}$. The ROS source could be cytochromes P450 upon detoxification of dihydrofusarubine. Besides, the latter generates $O_2^{\cdot-}$ and $OH^{\cdot}$ in the presence of dithiols [38].

Toxins (in particular, *Alternaria* tentoxin and *F. solani* isomarticin) can activate oxygen indirectly by uncoupling photophosphorylation, which finally leads to ROS formation in the first photosystem [38]. Culture filtrate from *Bipolaris sorokiniana* provokes (by a yet unknown method) H_2O_2 accumulation in barley leaves, which promotes infection [42].

Though toxins are pathogenic factors, their action can be the reverse, they can support not the disease but the development of resistance to it. Consequences of their activity are exactly the same as for elicitors, and their mechanisms also may involve oxygen activation. As an example, α-picolinic acid, one of the toxins of the causative agent of rice blast disease, is secreted by germinating spores, and in high concentration (1-3 mM) suppresses their germination [43]. This fungi toxicity is partly depressed by catalase, which points to dependence of the process on H_2O_2 [44]. α-Picolinic acid enhances H_2O_2 generation in contact with rice leaves, causes programmed cell death, and activates some plant genes connected with disease resistance. These plant reactions are evidently directed against the parasite, as leaf spraying with picolinic acid induces their resistance to subsequent infection by a virulent strain of *M. grisea* [45]. The mechanism of oxygen activation by picolinic acid is unknown, and is possibly linked to the presence of metal ions. The complex of nicotinic acid with Fe^{3+} effectively catalyses the Haber–Weiss reaction [46].

Tenuazonic acid, another toxin of the causative agent of rice blast disease (as well as fungi of genus *Alternaria*), provokes apparent symptoms of disease on rice leaves. However, they appear as small necrotic spots similar to ones arising after the infection of resistant breeds. If tenuazonic acid is applied to leaves together with spores, frequency of infection droplets of compatible type decreases. Namely, the toxin induces a local disease resistance. Tenuazonic acid is not toxic for spores in effective concentrations but diffusates of the treated leaves acquire toxicity. On addition of exogenous antioxidants, the latter decays, which links the fact to generation of ROS by spores [25].

Thus some elicitors or toxins, existing in the cell of pathogenic fungus or excreted to the extracellular medium, subject the host and the parasite to oxidative stress. The biological effect of this phenomenon can be quite the reverse, that is, it can either promote the infection or oppose it.

Oxidative burst apparently accomplishes its anticontagious functions only at the early stages of infection locally in infected tissue. Late abundant ROS production is known, higher in susceptible plants, which does not prevent tissue colonization by the parasite [47, 48]. It is possible that if strong oxidative damage at an early disease stage is insufficient to arrest the parasite, its subsequent development is already less sensitive to this factor. It is not inconceivable that insufficient oxidative burst promotes parasite antioxidant systems, increasing its tolerance to subsequent oxidative stress.

All this seems logical for biotrophic pathogens unable to use dead cells for food. There are suggestions, however, that for necrotrophic ones, *Botrytis*, for example, necrosis of plant tissues as well as oxidative burst leading to it were unambiguously favorable [49].

There are, however, opposing facts: $O_2^{\cdot-}$ production by bean leaves infected with *Botrytis* is enhanced 2-3 times stronger with *B. fabae* nonpathogenic to plants, than with the pathogenic strain *B. cinerea* [50]. Infection of suspension culture of bean cells with *B. cinerea* spores promotes generation of $O_2^{\cdot-}$ and H_2O_2. This capacity exhibited by a lowly aggressive fungal stock is several times stronger than in a highly aggressive one [51]. Resistance of tomatoes induced by the flavonoid *o*-hydroxyethylorutin to *B. cinerea* is coupled to an increased H_2O_2 production by leaves [52, 53]. Examples cited with *Botrytis* show that oxidative burst in an infected plant manifests itself more strongly if the plant resistance to the disease is higher, and, apparently, favors it. Just these typical necrotrophic fungi behave compatibly to biotrophes.

As described before, generation of $O_2^{\cdot-}$ and H_2O_2 in infection droplets on rice blast disease infected rice leaves enhanced mainly on account of the fungus. This, however, is hardly probable for the disease progression, as in the most studied incompatible combinations ROS generation was higher than in compatible ones [25].

Thus ROS on numerous occasions determine the interrelation of phytopathogenic fungus and the plant host, with the majority of cases as a factor increasing plant resistance to the disease.

OTHER FUNCTIONS OF ROS PRODUCED BY FUNGI

In some cases, ROS play a positive role in the organisms that produce them, in particular, via providing destructive energy. For instance, ROS, and, particularly, hydroxyl radical—the most reactive one produced—serve to disrupt cellulose and lignin in wood-degrading fungi

[54]. $OH^{\cdot}$ formation was registered via chemical and other methods in eight of ten tested fungi; this process was the most dramatic in *Antrodia vaillantii*, a causative agent of brown rot of wood [54, 55]. It is pertinent to note that $OH^{\cdot}$ generation is altered upon interaction of these fungi with bacteria. It is enhanced in the fungus *Antrodia vaillantii* by contact with antagonistic *Pseudomonas fluorescence* but it does not change on contact with *Bacillus subtilis*. In the fungus *Coniophora puteana*, $OH^{\cdot}$ generation was increased upon contact with either of these bacteria. It is supposed that wood-degrading fungi generate $OH^{\cdot}$ to attack bacteria. There is information that these fungi use the bacteria as nutrient substrates [55]. It is easy to suppose that phytopathogenic fungi also produce $OH^{\cdot}$ to penetrate the cell wall of the plant host.

Along with higher plants protecting themselves from parasitic microbes, microbes may use these weapons against competitive microflora. The fungus *Talaromyces flavus*, excreting the H_2O_2 producing enzyme glucose oxidase, suppresses growth of the fungus *Verticillium dahliae*, and thus protects eggplants from *Verticillium*-caused wilt. A strain of *T. flavus* producing 50 times less glucose oxidase does not protect plants from the disease [30, 31].

Antimicrobial properties of glucose oxidase, depending on H_2O_2, are well revealed upon transfer of the fungal gene encoding this enzyme to higher plant genome. Thus, tubers and leaves of transgenic potato plants produce H_2O_2 constitutively several times over the control ones. Transgenic plants are more resistant to bacterial soft rot, phytophtorosis, and *Verticillium*-caused wilt. Catalase suppression of resistance of transgenic plants infected by bacteria suggests the role of H_2O_2 in the plant defense against the disease [56].

It is conceivable that fungal antibiotics accomplish their functions also via oxygen activation. In any event, the participation of ROS in cytotoxicity of bacterial antibiotics has been repeatedly shown [57, 58].

ROS REGULATE GERMINATION, DEVELOPMENT, AND INTERCELLULAR COMMUNICATIONS IN FUNGI

ROS dualism, namely, their ability to be either harsh damaging factor or delicate regulators depends, in particular, on their concentration. The results of experiments wherein the concentration dependence of toxic H_2O_2 action on germinating spores of *M. grisea* has been studied were consistent with this dualism. It was established that the dependence was not monotonic over a wide range of H_2O_2 concentrations. On decreasing concentration from 10^{-2} to 10^{-5} M, toxicity decreased from almost complete suppression of spore germination to complete disappearance of the effect of H_2O_2. It is remarkable that highly diluted H_2O_2 solutions again showed toxicity. This property was revealed even at 10^{-12} M H_2O_2 and disappeared subsequently at 10^{-14} M [59]. Suppression of the spore germination by high H_2O_2 can be easily explained by the severe cell damage, but this is unlikely for its very low concentrations. It seems likely that H_2O_2 suppressed the germination at a regulatory level in the second case. Lack of the effect at 10^{-5} M H_2O_2 possibly means that such concentration was insufficient for immediate damage but it was too high for participation in regulatory mechanisms.

Recognition of self and alien species upon hyphal contact appears to by a crucial point in development of mycelial fungi. Silar analyzed H_2O_2 generation by contacting mycelia in a large group of fungi. This activity increased upon interaction of *Coprinopsis cinerea* and *P. anserina*. Enhancement of activity was not observed upon their interaction with other fungi. Increase in activity was also not observed upon the contact of the colony of one of these types with initially nonliving material—killed hyphae or yeast. It is of interest that the same reaction was provoked by some bacteria. It has been concluded that H_2O_2 serves as one of the signals for identification of alien organisms by hyphae [60].

It is of interest that ROS might serve as regulators of development of fungal population depending on its density. It is known that fungal development is inhibited not only in excessively dense spore suspensions, but also in overly diluted ones [61]. Self-suppression of spore germination in dense spore suspension could be easily explained by deficiency of vital resources and self-intoxication by vital activity products. Similar effects were observed in spore suspensions of *M. grisea*. It turned out unexpectedly that upon too high or extremely low spore concentrations, self-suppression of fungal development was decreased or completely eliminated upon addition of an antioxidant. Enzymes superoxide dismutase (SOD) and catalase or $OH^{\cdot}$ scavengers (formate and thiourea) possess these properties. Denatured enzymes, albumin, or urea, ineffective as antioxidants, did not have protective capabilities. The results obtained lead to the conclusion that germinating spores excrete ROS, which suppress spore development. Excretion of ROS by spores was supported by chemical analysis [24].

A dominating role of ROS in self-regulation of fungal development is surprising, but many other exometabolites, theoretically, can act in a similar way. ROS-promoting compounds presumably excreted from spores as diffusates from tightly compiled suspensions, or inversely, from dilute ones (but not optimal for germination) provoked toxicity inhibited by antioxidants in the other spores. Although poorer spore germination upon their overabundant concentration is easily to explain as being due to excess ROS, to understand the situation in a dilute spore suspension is more difficult. Most likely the biological effect depends not only on increase in ROS level but on its balance with antioxidants. The deficiency

of the latter presumably resulted in the inhibition of development in dilute suspensions.

The mechanism of oxygen activation by spores is obscure. Picolinic acid, secreted by spores, theoretically may serve as inhibitor of their germination [43], yet it was not observed in spore diffusate either under optimal spore concentrations or under increased concentrations [44]. At the same time, self-regulation of spore development can serve as a positive factor for the causative disease agent if it suppresses useless spore germination under conditions unfavorable for infection.

CHANGES IN ROS CONCENTRATION AND DIFFERENTIATION

High reactivity of ROS is responsible for oxidation of proteins, lipids, and nucleic acids. Consequently, systems defending against ROS by repair or resynthesis of damaged molecules are present in the cell. Availability of nutrient resources allows restoration of the cost of ROS neutralization. Nevertheless, impairment of intracellular redox status, as a result of an increase in generation of oxygen radicals exceeding the cellular capacity to neutralize them, can generate a hyperoxidation state (oxidative stress). As distinct from growth and differentiation state, oxidative stress is an unstable one, and elimination or partial inhibition of intracellular antioxidant systems may cause cell death [62].

Intracellular ROS increase is accompanied by the cessation of growth, and it provokes morphological changes leading to cell adaptation to changes in life conditions as well as the decrease in intracellular oxidants. Numerous experimental data support the relationship of triggering of differentiation processes with an increase in intracellular ROS concentrations [10, 62, 63]. Just so, in the myxomycete *D. discoideum* an increase in intracellular $O_2^{\cdot-}$ concentration as well as extracellular one provoked aggregation of myxamoebae and subsequent differentiation, and the aggregation process was prevented by $O_2^{\cdot-}$ scavengers together with an increase in expression of genes controlling antioxidant defense systems (ADS) [12]. At the onset of different stages of *N. crassa* macroconidia differentiation, a spontaneous, low-level chemiluminescence was detected enhanced by lucigenin and/or luminol, indicative of an increase in level of intracellular oxygen radicals. Antioxidants abolished chemiluminescence and stopped differentiation, which supports the formation of ROS ahead of every stage of fungal development [19].

H_2O_2 is considered as one of the most important metabolites in all respiring cells. H_2O_2 provoked global changes of gene transcription, including the ADS genes, in *A. nidulans* [63], as well as sclerotial differentiation in *Sclerotium rolfsii* [18], increased expression of genes of carotenogenesis in *N. crassa* [64], and promoted transition to filamentous growth in *U. maydis* and development of its pathogenicity [32]. It is known that sclerotial differentiation in *S. rolfsii* is coupled to H_2O_2 generation inside the cell. Its concentration increased under the action of light and iron ions [18].

$OH^{\cdot}$ formed on the interaction of transition metals with H_2O_2 was inhibited by such scavengers as dimethyl sulfoxide, phenylthiourea, *p*-nitrosodimethylaniline, ethanol, and benzoate, which suppress sclerotial differentiation in *S. rolfsii* [20]. Sclerotial differentiation was similarly inhibited by antioxidants (ascorbic acid, β-carotene) [65, 66]. It was shown that $O_2^{\cdot-}$ increased cleistothecium differentiation in *A. nidulans* [9], while $NO^{\cdot}$ promoted fruit body development in *F. velutipes* [15]. Addition of pheromone α to yeast cells with pheromone *a* causes ROS formation in *S. cerevisiae* cells. ROS are also required for sexual reproduction in *Volvox* [68]. Thus ROS formation is essential for fungal, myxomycete, and algal differentiation.

CHANGES IN FUNGAL CELL METABOLISM UNDER ROS ACTION

An increase of oxidant level inside the cell inevitably causes the oxidation of organic molecules. It has been shown that differentiation of sclerotia on the mycelium of *S. rolfsii* was accompanied by lipid peroxidation [69]. Light and Fe^{2+} enhanced lipid peroxidation as well as the intensity of sclerotium formation [18], and lipid peroxides and aldehyde degradation products inhibited many proteins, affected cell differentiation and proliferation, and might promote apoptosis [70]. Oxidation of sulfhydryl groups in proteins upon ROS action promoted a change in activity of some enzymes, as an example, decrease in glycolysis enzymes and enzymes of protein synthesis decline have been observed, coupled to cessation of growth.

At the start of separate steps of macroconidium differentiation in *N. crassa*, mass protein oxidation and their subsequent degradation [71], release of iron ions upon oxidation of [Fe-S] clusters of enzymes, oxidation of intracellular NADP and NADPH, glutathione oxidation, glutathione disulfide excretion to the extracellular medium [72], synthesis of antioxidant enzymes [10], and ROS-dependent chemiluminescence [19] were the experimental evidence of hyperoxidant state. An increase in protein carbonylation by ROS of 2.5 to 4 times has been observed in different species of mycelial fungi: *Mucor racemosus*, *Humicola lutea*, *F. oxisporum*, *A. solani*, *Cladosporium elatum*, *Penicillium chrysogenum*, *P. brevicompactum*, *P. claviforme*, *P. roquefortii*, *A. niger*, *A. argilacceum*, *A. oryzae* [73]. Oxidative stress was accompanied by cessation of growth and severe metabolic changes directed towards decrease in primary metabolites (acetate, glucose) and synthesis of compounds participat-

ing in cell protection, for example, carotenoids, melanins, proline, and polyols [62]. Trehalose is of fundamental importance in defending yeast cells in oxidative stress [74].

An essential role of alteration in metabolism under the influence of ROS and development of resistance to stress factors is due to proteasomes providing selective degradation of oxidized proteins [75]. Different regulatory factors including transcription factors, while being modified in the presence of ROS, change substrate specificity and proteolytic activity of proteasomes [76]. It is of interest that in *S. cerevisiae* proteasomic ATPases participate in transcription of stress-inducible and other genes, without any impact on proteasome proteolytic activity [77]. Increase in the content of oxidized proteins upon senescence or under pathological processes is caused by a decrease in proteosome activity.

FUNGAL ANTIOXIDANTS AND THEIR BIOLOGICAL FUNCTIONS

It is generally accepted that antioxidants are an important ROS counterbalance and defend the organism from exceeding oxidative stress. This is extremely important for pathogenic microorganisms, as they are exposed not only to their own ROS but also to those produced by the host. If a parasite uses destructive energy of ROS for destruction of the host's tissues, it, just the same, must have a reasonably strong mechanism for self-protection. Fungi possess a defense mechanism from external oxygen (limiting its penetration) as well as intracellular systems of defense against ROS, which prevents elaboration of oxidative stress.

Enzyme systems. Superoxide dismutases (SOD) (EC 1.11.1.6), catalases (EC 1.15.1.1), glutathione- and ascorbic-dependent peroxidases, and transferases are the enzyme systems decreasing $O_2^{\cdot-}$ as well as H_2O_2 produced as a result of its catalytic transformation. Effective cell defense against $^1O_2$, forming upon the action of light, can be provided by carotenoids. Enzymatic ADS are characterized by high specificity toward ROS, different intracellular compartmentalization, and presence of metal ions as catalysts, for example, copper, zinc, manganese, iron, and selenium. ADS enzymes are imperative for the vital activity of cells.

Superoxide dismutases. SODs catalyze dismutation of $O_2^{\cdot-}$, producing H_2O_2 and O_2, and they are believed to be the first line of defense against oxidative stress in eukaryotic cells. Native enzymes are highly stable, standing up to 100°C for 1 min, and they are also resistant to wide range of pH. In fungal cells, as in all other eukaryotic cells, two types of SOD occur: Cu,Zn-SOD in the cytosol, encoded by the gene *sod-1*, and Mn-SOD in the mitochondria, encoded by *sod-2* [78]. A unique peculiarity of Mn-SOD is its high resistance to H_2O_2 and its high inducibility: the rate of its mRNA transcription doubles and its stability increases three-fold upon the action of ionizing radiation [4, 79]. An extra expression of Cu,Zn-SOD and Mn-SOD genes occurred under oxidative stress in the stationary growth phase in *S. cerevisiae* [80]. Upon increased gene expression of Cu,Zn-SOD and Mn-SOD the lifespan and resistance to oxidative stress also increase in the cells of *S. cerevisiae* [81]. Mutants in *sod-1* of *N. crassa* and yeast show hypersensitivity to redox mediators and to increased oxygen tension. However, unlike other fungi, *sod-1* mutants of *N. crassa* are less sensitive to UV, heat shock, and γ-irradiation, therewith increasing in the activity of mitochondrial Mn-SOD [78] together with an increase in formation of carotenoids [82]. Increase in SOD activity has been observed upon spore germination and transition to stationary phase in *N. crassa* [83], and in the fungus *F. decemcellulare* SOD activity doubled upon transition to the stationary phase [84]. An increase in SOD activity by 2.0-2.3 times in 12 species of filamentous fungi was observed upon action of the redox mediator paraquat increasing intracellular $O_2^{\cdot-}$ concentration [73]. SOD mutants of *N. crassa* were distinguished by lowered lifespan, reduction of sexual reproduction, and decreased ability for formation of conidia [85], which points to involvement of these enzymes in cell defense against ROS and their important function in regulation of development.

It has been shown that in *S. cerevisiae* Cu,Zn-SOD and Mn-SOD are required in the late stationary growth phase for protection of mitochondrial proteins against oxidative stress [86]. Resistance to H_2O_2 as well as resistance to compounds increasing $O_2^{\cdot-}$ inside the cell, such as menadione, is largely dependent on functioning of Cu,Zn-SOD and glutathione reductase in *Sch. pombe* [87]. One of the observed molecular forms of SOD was essential for virulence development in *C. albicans* [88], and irregular cytoplasm Mn-SOD was obtained upon transition to the stationary phase in this fungus [89]. The participation of $O_2^{\cdot-}$ in DNA damage suggests that these oxygen species possess mutagenic functions, and SOD plays the main role in preventing mutations provoked by it. This was supported by the fact that spontaneous mutations increase has been observed in SOD-deficient mutants of *N. crassa* [78]. Thus both enzyme forms, Cu,Zn-SOD and Mn-SOD, are essential for the normal life cycle in filamentous fungi and yeasts.

Catalases. Catalases are a group of homologous proteins—metalloenzymes decomposing H_2O_2 to water and molecular oxygen in different organisms. Like $O_2^{\cdot-}$, H_2O_2 is a product of aerobic metabolism, forming as a result of a number of enzymatic and nonenzymatic reactions. According to its structure, catalase is a tetramer containing a single heme per subunit. Subunit size varies within the range of 55 kD in *C. tropicalis* to 80-85 kD in *N. crassa* and *A. niger* [78].

The presence of more than one catalase form is typical of many organisms, whereby various catalases are dif-

ferently regulated, and some of them are coupled to the developmental cycle of organisms [90, 91]. Two catalase genes have been revealed in *S. cerevisiae*—catalase A localized in peroxisomes and cytoplasmic catalase T. There are four different catalases in *N. crassa* encoded by genes *cat-1*, *cat-2*, *cat-3*, and *cat-4* [92, 93]. Contrary to other fungi, catalase activity in *N. crassa* is localized in the cytoplasm but not in peroxisomes [93]. Cat-1 is a homotetramer, glycosylated, resistant to temperature increase and to denaturants, and preserves activity in the presence of molar concentrations of H_2O_2 [91]. Cat-1 is the main spore catalase in *N. crassa*, its activity in spores being 60 times higher than in the mycelium [83, 91]. High catalase activity is linked to its increased synthesis during maturation and germination of conidia. The *cat-1* mutants differed in spore viability under the influence of light [94]. The activity of the other *N. crassa* monofunctional catalase, Cat-3, increased at the end of the exponential growth phase and upon hyphal aggregation [91]. In a mutant in *cat-3*, increased formation of aerial hyphae and conidia has been revealed [95]. Expression of the two main monofunctional catalases on developmental staged and upon stress factors is manifested differently in *N. crassa* [92]. Cat-2 and Cat-4 possessed peroxidase activity and contributed minimally to general catalase activity in the stages of development. Cat-2 activity was greatly increased in mycelium upon heat shock. The molecular weight of Cat-2 was about 165 kD, much lower than molecular weight of Cat-1 and Cat-3 (315 and 340 kD, correspondingly) and catalases from other organisms [92]. Genes of two catalases, similar to *N. crassa cat-1* and *cat-3* genes, have been revealed in *A. niger* [90]. Upon H_2O_2 treatment, increase in catalase activity by 2-3 times in 12 fungal species has been shown [73]. Genes of the pathogenic fungus *Cryptococcus neoformans* encode four different catalases; Cat1 and Cat3 are present in spores, Cat2 in peroxisomes, and Cat4 in the cytoplasm [96].

Peroxidases are another group of heme enzymes participating in decomposition of H_2O_2, as well as of organic peroxides. Peroxidases are broadly distributed in plant tissues where they are present mainly in peroxisomes. A small amount of the enzyme exists in animal cells [4]. Peroxidase activity was revealed in *N. crassa* upon exposure to heat shock, H_2O_2, arsenic, ethanol, and cadmium [97, 98].

Glutathione peroxidases (Gpx). Three *GPX* genes, homologous to mammalian ones, have been observed in *S. cerevisiae* genome. Lack of selenium atoms in the composition of catalytic cysteines is a fundamental difference of yeast from mammalian Gpx [99]. Unlike the homologous enzymes in mammals, glutathione peroxidases of fungi are monomers and show low peroxidase activity in the presence of glutathione. Thioredoxins are electron donors for them [100]. Gpx1 is synthesized in response to carbon starvation and Gpx2 upon oxidative stress in *S. cerevisiae* [100]. Gpx3 functions as redox-transmitter for transcription factor Yap1, triggering gene expression of ADS in response to oxidative stress in yeast [101]. The *gpx3* mutants show increased sensitivity to peroxides, whereas *gpx1* and *gpx2* have no phenotypical differences from the wild type.

Gpx1 and Gpx2 from pathogenic fungus *Cryptococcus neoformans*, similarly to the yeast enzymes, do not contain selenium in the active center cysteines [102].

Glutathione reductase (Glr). Glutathione reductase reduces glutathione disulfide (GSSG) and thus supports the balance GSH/GSSG in the cell. A gene encoding glutathione reductase, *GLR1*, was identified in the cells of *S. cerevisiae*. In *glr1* mutants, increased level of GSSG and doubly decreased GSH [103], and a higher sensitivity to oxidants was distinguished [104], while a higher content of mitochondrial oxidized thioredoxin was found in comparison to the wild type [105]. In the phytopathogenic fungus *F. decemcellulare*, Glr activity and glucose-6-phosphate dehydrogenase activity, supplying NADPH, increased five- and sixfold, respectively, upon transition to stationary phase [106]. A considerable increase in Glr activity was registered in *A. nidulans* upon its treatment by cadmium salts [107].

Thiol peroxidases—thioredoxins, glutaredoxins, sulfiredoxins—decompose H_2O_2 and organic peroxides as well as catalyze reduction of oxidized cysteine residues in proteins. It is significant that proteins involved in transduction of ROS signals are substrates of thiol peroxidases. The occurrence of thiol peroxidases stabilizes the genome of eukaryotic cells [108-110]. Their peculiar place in the ADS is associated not only with their removal of various peroxides, but also with their participation in ROS signal transmission and their interaction with transcription factors [101, 109-111].

Thioredoxins are small (about 12 kD) thermostable proteins containing a definite sequence in the active center: Cys-Gly-Pro-Cys [101, 112]. Thioredoxins demonstrate antioxidant properties, and they are able to restore conformation to oxidized proteins [112, 113]. At first, thioredoxins were identified as hydrogen donors for ribonucleotide reductase, but later their role was revealed in proteins, forming disulfides in a catalytic center, for example, in yeast *S. cerevisiae* transcription factor Yap1, and transcription factors p53, NF-κB, and AP-1 in mammals [101, 112]. The participation of the thioredoxin system in prevention of ribosome aggregation has been shown [114]. Oxidized thioredoxin is reduced with NADPH and enzymes thioredoxin reductase 1 in cytosol and thioredoxin reductase 2 in mitochondria. *Saccharomyces cerevisiae* has two genes encoding cytosol thioredoxins, *TRX1* and *TRX2*, and also the *TRX3* encoding mitochondrial thioredoxin [112, 115]. Deletions of *TRX1* or *TRX2* genes were not lethal for *S. cerevisiae*, but the mutants showed a doubling in GSH and a seven-fold increase in GSSG, and they also demonstrated a hyper-

sensitivity to H_2O_2. Deletions of both genes *TRX1* and *TRX2* led to elongation of the S-phase of the life cycle, to reduction of G1 interval, and also to an increase in GSH (3.5-fold) and GSSH levels (70-fold) [103, 112]. Thr redox potential of mitochondrial thioredoxin was not coupled to the thioredoxin system of the cytosol; it remained high even upon oxidative stress, and also in mitochondrial thioredoxin reductase mutants. It has been shown that glutathione reductase, localized in cytoplasm as well as in mitochondria, took part in the reduction of the mitochondrial thioredoxin [105]. In the pathogenic fungus *Cryptococcus neoformans*, shut-off of the thioredoxin reductase gene caused the loss of pathogenicity [102].

Thioredoxins are also specific electron donors for many peroxiredoxins. A constitutive activation of Yap1 is exhibited in the cytosol of yeast strains carrying mutations of all the three genes of the thioredoxin pathway, and, in comparison to the wild type, an increase in some Yap1-dependent proteins and peroxiredoxins: thiol specific antioxidant (Tsa1), stress inducible thioredoxin peroxidase (Tsa2), alkyl peroxide reductase (Ahp1), Cu,Zn-SOD, and a protein-chaperone delivering copper ions for Cu,Zn-SOD (Ccs1) were found. Besides, an increase in thiol oxidation in mutants compared to the wild type has been shown for such proteins as Cu,Zn-SOD, Ccs1, proteinase A (Pep4), methionine sulfoxide reductase (Mxr1), protein disulfide isomerases, Tsa1 and Tsa2, Ahp1, mitochondrial peroxiredoxin, and glutathione peroxidase 2 [116]. Upon H_2O_2 treatment, formation of protein disulfides in these proteins was higher in the yeast mutant strains of the thioredoxin pathway than in the wild type, which points to the significance of the thioredoxin pathway in detoxication of endogenous H_2O_2.

Glutaredoxins are structurally and functionally similar to thioredoxins. These proteins usually serve as antioxidants in yeast cells [117, 118]. Dithiol and monothiol glutaredoxins are differentiated according to the number of cysteine residues participating in catalysis. Most fungi contain monothiol as well as dithiol glutaredoxins [112, 117]. Reduction of glutaredoxins occurs with the participation of GSH, whose subsequent reduction is catalyzed by glutathione reductase in the presence of NADPH [112]. Cells of *S. cerevisiae* contain two dithiol glutaredoxins (1 and 2) homologous to bacteria and animal proteins, whose active center contains the sequence Cys-Pro-Tyr-Cys. Deletion of genes *GRX1* and *GRX2* provoked a decrease in yeast thermo tolerance, and an increase in their GSH (by 2.5 times) as well as GSSG (by 8 times) in comparison to the wild type [103]. Glutaredoxins 1 and 2 are localized in the cytosol and catalyze decomposition of H_2O_2 and hydroperoxides [118]. Glutaredoxins possess glutathione-S-transferase activity and play an essential role in formation of mixed disulfides of proteins with GSH upon the action of oxidants [119]. The reaction is reversible, and it is regulated by GSH level [119]. Glutaredoxin 2 was also found in mitochondria [112].

Three genes encoding monothiol glutaredoxins (*GRX3*, *GRX4*, and *GRX5*) have been revealed in *S. cerevisiae* [117, 120]. The catalytic center of monothiol glutaredoxins is characterized by the sequence Cys-Gly-Phe-Ser [117]. Glutaredoxins 3 and 4 are localized in the nucleus and glutaredoxin 5 in mitochondria. In mutants in *grx5* a defect in reduction of disulfide bonds was observed [112, 117, 121].

Peroxiredoxins are a class of antioxidant proteins reducing hydroperoxides to alcohols. These proteins possess peroxidase activity towards H_2O_2, peroxynitrites, and many of organic hydroperoxides. Thioredoxin peroxidases and alkylhydroperoxide reductases are related to them. They are localized in the cytosol and in the mitochondria. Also, peroxiredoxins associated with the nuclear and plasma membranes have been revealed. In cells of eukaryotes peroxiredoxins are present in considerable amounts and different isoforms, and in spite of their low activity in comparison to glutaredoxins and catalase, they apparently contribute a certain contribution to defense of cell plasma membranes against lipid peroxidation [122]. Along with catalases, these proteins play an important role in H_2O_2 detoxication in yeast [116, 123].

Three types of peroxiredoxins are distinguished according to the number of cysteine residues participating in catalysis, and the mechanism of their subsequent reduction: typical 2-Cys-peroxiredoxins, atypical 2-Cys-peroxiredoxins, and 1-Cys-peroxiredoxins [116, 122]. In all three types of peroxiredoxins the mechanism of peroxidase reactions is the same, and it includes oxidation of an active center cysteine to sulfenic acid upon simultaneous reduction of the substrate—peroxide [122]. Arginine is present in active centers of all the peroxiredoxins, essential for stabilization of the thiolate form of cysteine in the active center [122]. Subsequent reduction of oxidized peroxiredoxin arises differently. In the prevailing type of 2-Cys-peroxiredoxins there are two active cysteines per molecule, in the region of 50th (oxidized to sulfenic acid) and 170th amino acid residue. Typical 2-Cys-peroxiredoxins form dimers with two identical active centers. Oxidized cysteine attacks the dimer at the C-end of another subunit, forming a intermolecular disulfide bond. Atypical 2-Cys-peroxiredoxins are monomeric, and the oxidized cysteine of the active center undergoes a condensation reaction with the other cysteine, forming an intramolecular disulfide bond. Thiol compounds—thioredoxins (such peroxiredoxins are called thioredoxin peroxidases), glutaredoxins, GSH, and sulfiredoxins serve as electron donors for subsequent reduction of oxidized peroxiredoxins in the cell [116, 124]. Oxidized cysteine of 1-Cys-peroxiredoxin forms a mixed disulfide with the compound reducing it (glutathione-S-transferase, or directly with GSH) [122, 125]. 1-Cys-peroxiredoxin can be reduced by ascorbic acid [126].

Five genes of peroxiredoxins have been revealed in *S. cerevisiae*—*TSA1*, *TSA2*, *AHP1*, nuclear *TPX* (or *DOT5*), and *PRX1*, the first four being 2-Cys-peroxiredoxins and the last being a 1-Cys-peroxiredoxin [127]. Peroxiredoxin Tsa1 (thiol-specific antioxidant) defends glutamine synthetase from oxidative inactivation, it is present in all growth stages, shows higher affinity for H_2O_2 than for organic peroxides, and apparently it is the main peroxiredoxin in the cytoplasm. Tsa1 displays its antioxidant properties upon mitochondrial dysfunction. Insufficiency of Tsa1 in yeast causes an increase in SH-groups and activation of transcription of the main H_2O_2-dependent genes [128, 129]. Insufficiency of zinc causes an increased expression of Tsa1 [130]. In addition to peroxidase activity, Tsa1 accomplishes chaperone functions as well, which is of importance under oxidative stress and heat shock. Under normal conditions Tsa1 monomers in the cytoplasm form complexes with molecular mass about 272 kD, showing peroxidase activity, while appearing complexes of molecular mass about 545 kD display chaperone functions [129].

Induction of *TSA2* is observed under oxidative stress. In the presence of oxidants change in molecular mass and quaternary structure of cytosol peroxiredoxin Tsa2 as well as Tsa1 is coupled to changes in functional activity [131].

Disruption of the peroxiredoxin gene *AHP1* (alkylhydroperoxide reductase) does not influence vitality of *S. cerevisiae* growing in medium with glucose, but increases its sensitivity to organic peroxides. Participation of Ahp1 in yeast defense against toxic metals (copper, cobalt, chromium, arsenic, lead, and zinc ions) has been shown [132]. Ahp1 activity increased in Tsa1 mutants [131]. It is assumed that Ahp1 defends plasma membrane from oxidation in the presence of exogenous copper ions [133].

Nuclear peroxiredoxin was first cloned as one of the *DOT* genes (disrupter of telomere silencing). The two activities (the peroxidase one and regulation of gene expression) developed independently [134]. Nuclear peroxiredoxin displayed alkyl peroxidase activity and was required in the stationary growth phase [135]. It did not replace cytosolic peroxiredoxins and did not influence redox modification of Yap1 [134]. Cys106 in the nuclear peroxiredoxin molecule is the primary catalytic center, participating in creation of a disulfide bond with thioredoxin [135].

The *PRX1* gene encodes mitochondrial 1-Cys type peroxiredoxin [135]. Induction of *PRX1* was observed under the influence of H_2O_2 and hydroperoxides as well as at low glucose concentration [136]. As distinct from peroxiredoxins of cytosol, activity of mitochondrial peroxiredoxins is pH-dependent, and it sharply increases upon acidic pH shift, which is observed on activation of respiration with lowering glucose concentration in the medium upon transition to the stationary growth phase. It is assumed that the main function of the mitochondrial peroxiredoxin is the detoxication of ROS formed in the process of respiration [136].

Sulfiredoxins, thiol-containing ATP-dependent proteins (molecular mass about 13 kD), were first observed in yeast and then in bacteria and mammals. Sulfiredoxins reduce the sulfinic group ($-SO_2H$) to the sulfenic group (-SOH) in catalytic cysteine residues of typical 2-Cys peroxiredoxins, subsequent reduction of which to thiol group is accomplished by thioredoxins [108]. Peroxiredoxins and sulfiredoxins participate in H_2O_2 signal transduction in the yeasts *S. cerevisiae* and *S. pombe* [111, 116, 123].

Alternative oxidase. One of the fungal reactions in response to stress factors is increased cyanide-resistant respiration associated with appearance of alternative oxidase in the mitochondria [137, 138]. The enzyme is specifically inhibited by derivatives of benzhydroxamic acid [84, 106]. An alternative respiratory pathway branches from the main phosphorylating respiratory chain at the level of ubiquinone and transfers electrons directly to oxygen with formation of water molecules. Similarly to cytochrome oxidase, the reaction proceeds through a four-electron mechanism, without intermediate formation of oxygen radicals or H_2O_2. Transfer of electrons with the aid of alternative oxidase is not coupled with ATP synthesis, the significance of the reaction being competition with autooxidation of electron carriers with promotion of ROS [139]. Such a danger is extremely high upon blockage of electron transport, leading to accumulation of reduced electron carriers. As mentioned above, some fungicides are just such blockers [140]. Thus, resistance to them is often coupled to activation of alternative oxidase, which, for example, was observed upon exposure of *M. grisea* to the fungicide SSF-126. Activation of alternative oxidase in the same fungus by H_2O_2 suggests that oxidative stress was the basis of such fungicide action [141]. Under oxidative stress, alternative oxidase appears to be a unique terminal oxidase promoting fungal growth. Alternative oxidase is activated in fungi upon glucose depletion in the medium, upon transition to the stationary phase, as well as under oxidative stress, lowering the activity of the cytochrome pathway [84, 106, 138, 142]. Increase in alternative oxidase activity has been demonstrated in many industrial fungi [73, 143].

Nonenzymatic antioxidants. Various antioxidants—ascorbic acid and its derivatives, glutathione, proline, trehalose, polyols, tocopherols, as well as pigments such as carotenoids and melanins—are present in fungal cells.

Ascorbic acid. It is known that ascorbic acid restores the antioxidant properties of vitamin E (tocopherol), thus it appears as an indirect lipid antioxidant [4]. The implication of these compounds in fungal differentiation has been demonstrated in *S. rolfsii*, *Sclerotinia sclerotiorum*, and *S. minor* [65, 66, 144]. A twofold decline in the amount of reduced ascorbic acid was observed during differentiation of these fungi. Addition of exogenous ascor-

bic acid lowered lipid peroxidation in fungal cells and inhibited sclerotial differentiation.

Glutathione. The most ubiquitous thiol in cells—the tripeptide γ-L-glutamyl-L-cysteinyl-glycine—is present in a reduced form (GSH) and two oxidized ones, glutathione disulfide (GSSG) and mixed disulfide of glutathione with proteins (GSSR) [145]. Interruption of GSH biosynthesis in yeast *S. cerevisiae* decreased their resistance to oxidative stress [146]. GSH plays an important antioxidant role in cells by decreasing ROS level [79]. Intracellular GSH concentration in yeasts varies from 5 to 20 mM [104]. Owing to the fact that GSH concentration in yeast cells under physiological conditions surpasses GSSG concentration by 10-100-fold, protein thiols are presumably reduced in the cell [113]. High cellular GSH level is supported by NADPH-coupled glutathione reductase, and thus it is tightly linked to the presence of oxidizable substrates in the cell. Intracellular redox status shifted towards higher reduction level often provides cell survival in extreme conditions. Defense of redox-sensitive SH-groups in protein cysteine residues against irreversible oxidation upon ROS increase is achieved by forming GSSR, and thereat a reversible inhibition of the activity of some enzymes was observed, turned off by recovery of the cell redox status [113, 121]. In *S. cerevisiae* mutants unable to synthesize GSH, introduction of H_2O_2 caused an irreversible cysteine oxidation in some glycolysis enzymes as well as enzymes of protein biosynthesis [121].

Apart from support of intracellular redox homeostasis and direct antioxidant defense function, GSH participates in the work of antioxidant enzymes as a cofactor [147]. Another antioxidant glutathione function was revealed—detoxication and transport of intracellular copper [104, 148]. With the involvement of cysteine group, GSH is able to deliver copper to apoproteins of copper-containing proteins including Cu,Zn-SOD [148]. These functions promote binding of intracellular copper, thus preventing potentially toxic reactions between metals and oxygen (Fenton reaction). Besides, cysteine excess appears to be toxic for the cell, and glutathione provides the cell with a nontoxic form of cysteine [145].

Metallothioneins. This is a widespread class of cysteine-rich proteins (containing 30% cysteine) of small size (<7 kD). They support metal homeostasis in the cell, as despite the important role of metal ions in catalysis and regulatory functions of intracellular proteins, concentration of metal ions has to be maintained within certain limits to prevent their toxic action. These proteins are present in prokaryotes as well as in eukaryotes. Together with regulation of metal ion homeostasis, metallothioneins possess some physiological functions—they are detoxifiers of metal ions, scavengers of free radicals, and regulators of growth and proliferation [149].

An increased content of cysteine, serine, lysine, and aromatic amino acids as well as a high affinity for metal ions is an attribute of metallothioneins. Along with metal ion transfer in fungi and yeast, metallothioneins sequester copper, zinc, cadmium, mercury, and silver ions [104]. They are encoded by two genes (*CUP1* and *CRS5*) in the yeast *S. cerevisiae* and protect the yeast cell against oxidizing agents. In an *S. cerevisiae* cells with a mutation in Cu,Zn-SOD, an increased expression of metallothioneins was observed. The *CUP1* gene was also induced on exposure of yeast cells to menadione [104].

Metallothionein structure has been recently established in *N. crassa*. Synthesis of mRNA of this protein was induced *in vivo* only with copper ions; however, the protein is able to bind *in vitro* other mixed-valence metals—zinc, cadmium, cobalt, and nickel [150]. Moreover, the metallothionein gene *MMT1* was identified in the parasitic ascomycete *M. grisea* causing rice blast disease. It is actively expressed during growth and development of the parasite and encodes a protein containing 22 amino acid residues including six cysteine residues. The protein shows a high affinity toward zinc and possesses high antioxidant activity. In case of mutation in this protein, appressoria (specific organs of penetration in fungi) are unable to penetrate the cuticle of the host cells. Besides, metallothioneins have been found in *Agaricus bisporus*, and recently in mycorrhizal fungi [149]. In *S. cerevisiae*, *N. crassa*, and *C. glabrata* existence of phytochelatines—peptides increasing fungal tolerance to metal ions—has been shown [151].

The concentration of $O_2^{\cdot-}$, H_2O_2, GSH, as well as catalase activity was increased in the yeast *Candida intermedia* in the presence of copper ions, and tolerance to metal ions depended on the rate of ROS generation and ADS effectiveness [152]. Addition of magnesium ions to the growth medium of *F. acuminatum* and *F. equiseti*, in spite of increase in activity of Mn-SOD and catalase, promoted lipid oxidation and increased iron ion concentration, apparently coupled to ROS formation in the Fenton reaction [153]. Metal chelators acted as antioxidants by binding metal ions.

Scavengers of hydroxyl radical. Sugar alcohols, e.g. mannitol and arabitol, which are abundant in fungal cells, also apparently play an antioxidant role. Spores of wheat stem rust contain about 10% of these sugars in relation to total cell weight [154]. Spores of rice blast disease causative agent contain relatively similar amounts of mannitol. It is assumed that mannitol functions as an osmolyte. It is not used for spore germination [155].

Mannitol is widely used in the laboratory as an effective $OH^{\cdot}$ scavenger. In fact, in the pathogenic for people fungus *C. neoformans* (synonym *Filobasidiella neoformans*) a mutant with lowered concentration of mannitol was more easily attacked than the wild type by cytotoxic neutrophils and by hydroxyl-generating model system. In both cases, exogenous mannitol protected the fungal cells [156].

Jennings and coworkers have proposed that mannitol of phytopathogenic fungi inhibited hydroxyl-dependent

phytoimmune reactions [157]. However, plants contain the enzyme mannitol dehydrogenase, which oxidizes mannitol to mannose. In sterile tobacco plants, only traces of mannitol dehydrogenase are present, but its activity increases upon contact of the plant with fungi. On the other hand, on contact of *Alternaria* culture with the extract from the plant, mannitol concentration, excreted by the fungus, increased.

Jennings's arguments are true only if mannose is a weaker antioxidant than mannitol. Lack of significant differences between mannose and mannitol in their ability to scavenge $OH^{\cdot}$ goes against it. Both compounds equally defended spores of *M. grisea* from fungal toxicity of model hydroxyl-generating system, as well as from ROS-dependent toxicity of excretions of rice leave [158]. However, it is significant that mannose is easily metabolized in the cell, and intracellular mannitol concentration remains relatively constant.

Carotenoids. The results of a series of investigations on fungal cells point to a relationship between carotenoid synthesis and cell protection against the photodynamic effect of ROS produced in the presence of light and oxygen [159-161].

β-Carotene, neurosporaxanthin, and astaxanthin are the major carotenoids found in fungi. It has been shown that regulation of carotenoid biosynthesis in fungi is achieved by light of the blue-violet range of the visible spectrum, oxygen, and temperature of cultivation [162]. Nitrogen and glucose starvation provoke carotenoid biosynthesis in *N. crassa* [161]. Absence of Cu,Zn-SOD in *Blakeslea trispora*, *Phaffia rodozyma*, and *N. crassa* resulted in increased formation of carotenoids in them, thus supporting the importance of carotenoids as antioxidants [82, 163, 164]. ROS are shown to regulate carotenogenesis in *P. rhodosyma* and *F. aqueductum* [159, 163]. High sensitivity of fungal gene mutants of carotenoids biosynthesis to UV radiation points to ability of carotenoids to protect the spores from these factors [165]. Yet more differences in sensitivity to $^1O_2$ have been revealed in carotenoid-containing fungi and those not containing it. The yeast *Rhodotorula mucilaginosa* rich in the carotenoid torularhodine were more resistant to high concentrations of oxygen and duroquinone generating $O_2^{\cdot-}$ than nonpigmented strain of *S. cerevisiae*. Increased resistance to ROS was eliminated by the inhibitor of carotenoids synthesis diphenylamine, but it was restored upon addition of β-carotene [166].

Melanins. Black pigments—melanins—defend the fungal cell from various stress factors including ionizing radiation. Melanins of mycelial fungi are still poorly investigated, and the nature of precursors of their synthesis remains unclear. The properties of melanins depend on their chemical composition, the presence of various functional groups or radicals. Pigments synthesized by micromycetes belong to dihydronaphthalene type, and those synthesized by macromycetes—to pyrocatechol type [167]. It is significant that melanins are antioxidants. This apparently involves general-purpose of their defending properties, as oxidative stress is a universal part of damage caused by many extreme factors.

Melanins are localized in the cell walls of mycelium and spores [168]. Appressorium melanization provides rigidity of the contagious hypha in phytopathogenic fungi necessary to penetrate the plant cell wall [169]. Melanin-containing fungi are usually more resistant to extreme conditions than related nonpigmented ones [170]. For example, a pigment protects the cells from photodestruction, and this can easily be explained by a simple screening, but melanin protection against ionizing radiation and other damaging factors of non-radiation origin is more complex [170].

Mycelium and conidia of causative agent of rice blast disease contain melanin, making them dark gray. Voynova and coworkers produced nonpigmented (*alb-1*) mutants or mutants containing a defective rosy one (*ros-1*). These mutants were unable to attack rice plants susceptible to the parent strain [171].

Nonpathogenicity of melanin-deficient mutants apparently depends not only on defective processes of penetration into the host cell, but on sensitivity to abiotic environmental factors and to protective host reactions. Intense light suppressed spore germination in mutants and their appressoria formation more strongly than in the parent strain, and this damage was weakened upon addition of exogenous SOD and catalase to the medium [172]. Diffusates of rice leaves, toxic for the fungus, suppressed spore germination in mutants more strongly than the spores of the wild type [173]. Toxicity was weakened by antioxidants, which points to the participation of ROS in the process. Mutants were also extremely sensitive (in comparison to the parent strain) to any artificially promoted ROS—$O_2^{\cdot-}$, $OH^{\cdot}$, H_2O_2 [174], and $^1O_2$ [175]. Addition of melanin, isolated from the mycelium, weakened the spore damage by the leaf diffusive as well as by any of the ROS-generating model systems [173-175]. Endogenous melanin offers superoxide dismutase and catalase activities [174]. Ability to scavenge $^1O_2$ has been shown and for other melanins [176]. It cannot be excluded that defense from oxidative damage by melanins is due to their ability to bind metal ions that catalyze free radical reactions. In experiments with *M. grisea*, addition of exogenous melanin to spores after breakdown of ROS did not defend spores; thus, the protecting effect of melanins is rather determined by prevention of defects than by reparation of them [174].

Data show that melanin of *M. grisea*, and, presumably, of other phytopathogenic fungi, is essential for their parasitizing, particularly due to its antioxidant properties. As an example, parasitic forms of the zoopathogenic fungus *Wangiella dermatitides* contain in the cell wall five times more melanins than saprophytic ones, and they are more resistant to UV radiation [178].

Other compounds. Concentrations of oxalic acid secreted by phytopathogenic fungi of genus *Sclerotinia* were about millimolar. Mutants deficient in this acid lost the ability to provoke the disease, and the revertants assumed it again. Thus oxalic acid appears to be a pathogenic factor. This may be connected to its antioxidant properties, as pure oxalic acid or culture medium of *S. sclerotiorum* containing it suppressed oxidative burst in soybean and tobacco suspension cultures provoked by addition of elicitor [179].

Antioxidant activity coupled to pathogenesis is also shown by 2-methylsuccinate. This compound was isolated from bean leaves infected by *Botrytis cinerea*. Noninfected leaves revealed background $O_2^{\cdot-}$ level, but around infected tissue it was weakened. Intercellular fluid from the decolorized zone suppressed $O_2^{\cdot-}$ generation by other leaves, and the acting compound was identified as 2-methylsuccinate [51].

It has been reported recently that proline has antioxidant properties. In a mutant pathogen fungus of lucerne, *Colletotrichum trifolii*, spontaneous intensive H_2O_2 production was observed leading to apoptosis. Proline defended mycelium cells of *C. trifolii* from the action of UV radiation, heat and salt stresses, and H_2O_2, preventing apoptosis [180].

Thus the presence of antioxidants and of intracellular mechanisms of ROS deactivation gives fungi the possibility to survive and reproduce under the influence of various stress agents. It is significant that the level of antioxidants is under genetic control.

CHANGES AT THE CELL SURFACE

Decrease in surface area is one of the mechanisms of fungal mycelial adaptation to an increase in ROS in the environment. Indeed, upon oxidative stress adhesion of mycobacteria and of plasmodia in myxomycetes and hyphal aggregation in *N. crassa* and higher fungi occurs. Hyphal aggregation is one of the initial stages of differentiation of a fungal organism. It is of interest that usually the aggregation process is accompanied by biosynthesis of pigment molecules in the mycelium [62]. Such a hyphal "adhesion" reduced the entrance of oxygen inside the cell due to a decreased surface. Ability to aggregate has been revealed in hyphae of microscopic fungi of Chernobyl under increased radiation [181]. In higher fungi the result of such an adhesion is the formation of a fruit body. There are some other methods to decrease oxygen permeation into the cell, for instance, an increase in the viscosity of polysaccharides of the cell wall [62]. Most protected from oxygen penetration are the resting fungal spores provided with a thick cell wall as well as with a high level of low-molecular-weight compounds such as polyols, trehalose, proline, pigments, etc. Lowering of water penetration into the cell together with dissolved oxygen increases protection of intracellular structures.

Hydrophobins. The surface of asexual spores (conidia) of many fungal species is covered by a layer of tightly packed interweaved bundles of rodlets of 5-10-nm thickness composed of hydrophobins (GPh)—unique fungal proteins. This layer is formed by self-assembly of GPh monomers on the cell surface. GPh were originally discovered in the laboratory of Wessels in the basidiomycete *Schyzophyllum commune*, and they were found later in most fungal species [182, 183]. GPh are a new class of proteins possessing specific physicochemical properties [182, 183]. For GPh isolation, some atypical methods have been used. The hydrophobin family includes small secreted proteins (100 ± 25 amino acid residues) containing a typical N-terminal sequence of signal secretion, but conservative degree in amino acid sequences among them is not high [182]. Structurally, GPh are composed of two similar domains, each containing four cysteine residues that are involved in formation of intramolecular disulfide bridges. Typical GPh structure is the following: Cys-X_{5-9}-Cys-Cys-X_{5-18}-Gly-X_{5-20}-Cys-X_{8-23}-Cys-X_{5-9}-Cys-Cys-X_{2-12}-Gly-X_{3-10}-Cys-X_{2-13} [184]. A signal sequence and poorly conserved N-terminal segments precede the first cysteine of the first domain. Cysteine residues form four intramolecular disulfide bridges between cysteines 1 and 2, 3 and 4, 5 and 6, and 7 and 8, to produce two pairs of loops separated by a connector region of 8-23 amino acids [184]. Poorly conserved residues (X) are biased toward hydrophobic amino acids. The size of the second loop is more variable but contains at least one glycine residue that is usually adjacent to a hydrophobic amino acid. GPh are divided into two classes: class I hydrophobins assemble into aggregates that are stable to detergent and ethanol, whereas class II hydrophobins can be solubilized to monomers with these treatments [182].

It has been shown that GPh molecules are secreted to the medium via hyphal apex in depth culture, but form complexes not only at the cell wall–air interface but also at interfaces between water and any hydrophobic material [182]. Aerial hyphae and conidia, covered with GPh rodlets are difficult to wet with water because of the hydrophobic nature of the outwardly facing rodlet side of the hydrophobin layer, which promotes not only aerial hyphal growth but as well the distribution of spores in the environment, and apparently, serves as a barrier to oxygen diffusion to the fungal cell [182]. The interior of the hydrophobin layers contacting the hyphal wall is hydrophilic. Mycelium of mutants lacking the hydrophobin film is easily wettable, which complicates their growth in air. GPh monomers isolated from the rodlets can spontaneously assemble into an insoluble amphipathic film at the interface of hydrophilic and hydrophobic phases.

Genes encoding hydrophobins are found in different classes of fungi. They can be expressed at different stages

of the life cycle [161]. Hyphae assimilating nutrients are deprived of hydrophobins. They appear during ontogenesis and form a typical film on the surface of aerial hyphae, upon emerge of conidia, on hyphal adhesion in the process of fruit body formation of basidiomycetes, as well as in the process of attachment of hyphae of parasitic fungi to the host cell wall [182].

ROS SIGNAL TRANSDUCTION IN FUNGI

In the context of various fungal reactions to oxidative stress, the question arises: how does a fungal cell accept an ROS signal, and how is the response realized? In yeast this problem was investigated most completely, but signal transduction pathway elements appear to be similar in yeast, filamentous fungi, and animal cells [185, 186]. It has been shown that more than half of the *S. cerevisiae* genome is related to yeast adaptation to such stress factors as starvation, temperature shifts, oxidative stress, and variations in pH value and medium osmolarity [187]. As a response to different oxidants, specific changes in gene expression occur, including genes of the ADS [63, 187-190]. In response various oxidants not only ROS origin but their concentration as well influences the response of the organism, which points to specificity of ROS signal transduction.

The ability of ROS to interact with various organic molecules creates problems in identification of the molecular targets. It was revealed that receptor kinases, phosphatases, regulatory proteins, and membrane lipids appear to be the targets of ROS in the cell [3, 191, 192].

Thiol compounds play an important role in ROS perception. Immediate oxidation of protein SH-groups in the presence of ROS, diversity of the products produced (thiyl radical -S$^{\bullet}$, disulfide -S-S-, sulfenic -SOH, sulfinic -SO_2H, and completely oxidized sulfonic acid -SO_3H as well as formation of sulfenylamide or sulfinamide with the adjacent amino acid residues), and reversibility of these reactions make thiols the key compounds in reception and transduction of the ROS signal [193]. Ionization of certain protein SH-groups, caused by the influence of adjacent amino acid residues, increases their sensitivity to interaction with ROS by several orders of magnitude [193]. Formation of sulfenic acid in the presence of H_2O_2 upon the oxidation of GSH was recognized in catalytic centers of peroxiredoxins, glutathione reductase, methionine sulfoxide reductase, and protein tyrosine phosphatases [113, 193]. In a tyrosine phosphatase molecule the possibility of both has been shown: appearance of an intramolecular disulfide bond and formation of sulfenylamide with adjacent serine and lysine residues, together with a change in conformation and stability of the molecule [193]. As an example, oxidative modification of cysteine residues in transcription factor Yap1 upon H_2O_2 signal transduction regulated by glutathione peroxidase 3 (Orp1p) has been shown in yeast [116, 192]. Oxidation of cysteine residues in proteins provoked by ROS was revealed also in protein kinases and protein phosphatases [2]. Thus high reactive capacity and diversity and reversibility of oxidative modification enable sulfenic acid to serve as a ROS sensor in some enzymes and transcription regulators [194]. Other amino acid residues in proteins (methionine, histidine, tyrosine, and others) also appear to be ROS sensors [76, 195-197].

Under normal physiological conditions, protein sulfhydryl groups are predominantly reduced in the cytoplasm due to high GSH concentration [113]. SS/SH balance in proteins depends on oxygen. Preincubation of *S. cerevisiae* cultures under a nitrogen atmosphere reduced the overall amount of protein disulfides tenfold compared with aerobically cultivated yeast [113]. Two hundred proteins were revealed in *S. cerevisiae* upon aerobic cultivation, 64 of which were identified. H_2O_2 treatment (1 mM, 1 min) of yeast cells did not produce protein disulfide bond formation *de novo* but enhanced their production mainly in control proteins. Increase in disulfides, revealed upon H_2O_2 addition, was largely related to formation of mixed disulfides with GSH. Increase in mixed disulfides formation has been shown for alkylperoxide reductase, glyceraldehyde phosphate dehydrogenase, aldehyde dehydrogenase, pyruvate kinase, pyruvate decarboxylase, cystathionine-β-synthase, and inorganic pyrophosphatase. Thus specific proteins are oxidized upon ROS treatment, which presumably reflects their implication in reception and transduction of ROS signal [113]. Various ROS-sensitive enzymes with different cell functions, such as glycolysis enzymes and enzymes of protein synthesis, appear to be ROS sensors [113, 121, 196, 198]. Activity of the main respiratory chain (the main source of intracellular ROS) decreases in response to ROS, and electron flow is switched to the alternative oxidase [106, 143].

ROS signal transduction to the transcription factors in fungi might follow different pathways common to all living systems: two-component phosphorylation system (histidine/aspartate phosphorylation), G-proteins, mitogen-activated protein kinase (MAPK) cascade.

Homologous to the animal pathways, fungal G-protein pathways are used to sense and respond to environmental stimuli. A class of transmembrane fungal receptors (G-protein-coupled receptors, GPCRs), through their interaction with heterotrimeric G proteins, regulates the activity of second messengers and protein kinases and thus transduces pheromone and nutrient signals [191]. *Neurospora crassa* G-proteins are involved in ligand binding. They control growth rate, asexual reproduction, and stress resistance via regulation the activity of adenylate cyclase [199, 200]. Some G-protein mutants of *N. crassa* are sensitive to elevated temperature and H_2O_2 treatment [200].

The two-component phosphorylation system with a sensor module containing the PAS domain was applied in

a broad spectrum of cell responses to the environment [191, 201]. Fifteen histidine kinase genes, genes of four response regulators, and of a single histidine-containing phosphate transporter gene were detected in the *A. nidulans* genome [202]. Response regulators transfer the histidine kinase signal to a MAPK cascade (e.g. SskA in *A. nidulans*) or function as a transcription factor directly, like SrrA [202]. Investigation of histidine protein kinases is of interest as a target in regulation of fungal development. It has been shown, for example, that fungicides (iprodione and fludioxonil) provoked, through excessive activation of *A. nidulans* histidine protein kinase (NikA), an increased activation of the transduction pathway, leading to MAPK cascade activation, and to overexpression of conidium-specific genes *catA*, *gfdB*, and *tpsA* encoding catalase, glycerol 3-phosphate dehydrogenase, and trehalose. Growth arrest at early stages of development was the result, which was not observed after fungicide treatment of mutants in these genes [203]. Eleven histidine kinases are present in *N. crassa* transferring the signals to two response regulators [201].

At the molecular level in filamentous fungi, light-absorbing photoreceptor WCC complex of *N. crassa* controlling all the light responses was investigated. Amplification of expression of light-dependent genes of carotenoid biosynthesis via WCC complex has been shown as an example of the light action in the presence of H_2O_2 [64].

Direct interaction of transcription factors with the transcription cis-regulatory elements, nucleotide sequences of gene promoter regions, controls changes in gene expression. Yap1, Skn7, Msn2, and Msn4 are the most extensively studied transcription factors regulating stress response in *S. cerevisiae* [204]. The main transcription factor of Yap family, Yap1, regulates gene expression of antioxidant enzymes and components reducing the cell thiols. Owing to this, yeast retains resistance to such oxidants as *t*-butyl-hydroperoxide, diamide, diethylmaleate, and cadmium [101].

Skn7 induces an additional to Yap1 expression of not less than 15 proteins in response to H_2O_2 and *t*-butyl-hydroperoxide but not cadmium 97 [205]. Unlike Yap1, Skn7 is not involved in the regulation of metabolic pathways generating the main reducing cell compounds—glutathione and NADPH [205].

Msn2 and Msn4 induce the genes containing stress response elements (STRE, motif CCCCT) in the promoter as a response certain stress factors. Along with the Yap1-regulon common genes (coding eight proteins), Msn2/4 regulates a small number of antioxidant enzymes and several heat-shock proteins. It is also related to ubiquitin metabolism and protein degradation in proteasomes [206].

Studies of the last decade have shown that ROS affect all the aspects of fungal life. They promote post-translational conformation modifications of proteins and their activity, as dictated by specificity and intensity of the stress agents, influence metabolism and physiological functions, and regulate growth and development of fungi [113, 121, 195, 207]. The effect of ROS on the organism largely depends on the cell redox status, i.e. it is determined by redox potential and the redox capacity of the redox couples of the biological system [208].

Fungi represent a broad group of organisms that which can live as saprophytes, parasites, and symbionts. They differentiate in the process of development giving way to 15-20 various forms during the life cycle. ROS regulate all the vitally important processes in fungi: phase development change, intercellular communications, and protection from interspecies competition. Phytopathogenic fungi possess specific means of ROS production largely determining their interrelations with the host. Different sources of ROS in fungi as well as their detoxifying systems enable fungi to maintain a ROS level required for signaling. Regulation of ROS generation in the cell and of the activity of antioxidant defense systems is determined by the redox status of the cell and depends on intracellular as well as extracellular factors. Interactions of ROS with target proteins and reversibility of this interplay upon the change in oxidant concentrations underlie reception and transduction of the ROS signal as well as post-translational modifications of conformation and activity of the enzymes causing a change in cell physiology. Adaptation to environmental cues and organism survival in extreme conditions appear to be provided by cell efficiency in bringing into action ROS signal transduction rapidly and specifically.

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