Chapter 9
Unique Metabolic Responses to Hypoxia and Nitric Oxide by Filamentous Fungi

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Abstract To understand fungal metabolism under stress is important for the industrial production of organic acids and enzymes from fungi, and also for traditional large-scale fermentation because inadequately controlled cultures impose stress on fungi that reduces performance efficiency. This chapter describes recent advances in studies of the hypoxic regulation of fungal metabolism. Transcriptome and proteome analyses of the model filamentous fungus *Aspergillus nidulans* have identified global metabolic changes in carbon source utilization and energy conservation under hypoxia. Insufficient nitrate reduction under hypoxia results in the generation of nitrite or reactive nitrogen species (RNS) that constitute a prevalent nitrogen source for filamentous fungi such as *Aspergillus*. Fungi have developed novel nitrate reduction mechanisms to survive under hypoxia that are likely to be industrially important because they can generate RNS during fermentation. This chapter also describes recent findings of heme biosynthesis and nitrosothionein that are involved in fungal responses to RNS and detoxification mechanisms.

Keywords Ammonia fermentation • Denitrification • Heme • Hypoxia • Nitric oxide • Nitrosothionein • Proteomics • Transcriptomics

Abbreviations

GABA	γ-Aminobutyric acid
GSNOR	GSNO reductase
iNT	Nitrosothionein
Nar	Nitrate reductase
Nir	Nitrite reductase
Nor	NO reductase
PPP	Pentose phosphate pathway
RNS	Reactive nitrogen species
TCA	Tricarboxylic acid TrxR, thioredoxin reductase

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9.1 Industrial Applications of Filamentous Fungi

The production of East Asian wines that require filamentous fungi to saccharify cereal starch has been established for centuries, and such fungi have since become indispensable to the global production of industrial enzymes, organic materials, and pharmaceuticals. Hydrolyzing enzymes derived from various filamentous fungi are applied to detergent manufacturing, food processing, and the saccharification of starch and biomass cellulose. Some filamentous fungi are sources of antibiotics such as penicillins and cephalosporins, cholesterol-lowering statins, and other drugs. These applications depend on the fermentative properties of filamentous fungi that are supported by their unique metabolic activities. Culture conditions, namely nutrients, temperature, aeration, and pH, must be precisely controlled to maximize fermentation performance because inadequately controlled conditions impose stress on fungi, which in turn threatens the fermentation process and affects fungal survival. Thus, understanding fungal metabolism under stress is important for industrial applications of fungi.

The genus *Aspergillus* contains several industrially valuable species. *Aspergillus oryzae* is used to hydrolyze rice and soy beans during fermentation to manufacture Japanese rice wine (sake), soy sauces, and pastes. *Aspergillus niger* and *Aspergillus terreus* produce citric and itaconic acids that are useful in the food and chemical industries, respectively. All fermentation processes are affected by culture conditions as just described. Aeration is critical because most filamentous fungal species are absolutely aerobic, or require oxygen for growth, cellular metabolism, and hence fermentation efficiency. For example, *A. niger* produces less citric acid under low oxygen (hypoxic) conditions than under normoxic conditions. To understand the metabolic changes induced by hypoxia is therefore important for industrial applications, yet little is known about the biology of filamentous fungi under a hypoxic milieu.

This chapter briefly describes recent advances in studies of the hypoxic regulation of fungal metabolism. Transcriptome and proteome analyses of the model filamentous fungus *A. nidulans* have found that global metabolic changes occur in the utilization of carbon sources and energy conservation under hypoxia. More recent studies have found that hypoxia generates nitrite or reactive nitrogen species (RNS) because of the insufficient reduction of nitrate added to fermentation mixtures as a nitrogen source. This process results in disrupted metabolism and damaged DNA. Fungi have developed novel nitrate reduction mechanisms to survive in a hypoxic milieu. Such mechanisms are likely to be industrially important because they can generate RNS during fungal fermentation that includes nitrate, which is a popular nitrogen source for filamentous fungi such as *Aspergillus*. The following chapter describes recent findings of fungal responses to RNS and detoxification mechanisms.

9.2 Hypoxic Stress Responses of Filamentous Fungi

Most eukaryotes are obligate aerobes that require oxygen not only for the final electron acceptor of respiration but for biosynthesizing sterol, NAD, heme, and other metabolites (Bunn and Poyton 1996). Filamentous fungi as well as other eukaryotes were originally considered to be obligate aerobes, and when faced with a low-oxygen milieu, their growth was limited by oxygen-requiring metabolism. However, some fungi can survive very low (<0.5 ppm) concentrations of oxygen (hypoxic conditions) (Hall and Denning 1994). These findings suggested that fungi cannot be considered obligate aerobes in terms of energy conservation. This chapter describes these fungal hypoxic mechanisms and how filamentous fungi respond to oxygen availability and regulate their metabolic mechanisms at the cellular level.

9.2.1 Nitrogen Metabolism Under Hypoxia

Filamentous fungi can assimilate a diverse range of nitrogen sources. In addition to mechanisms of nitrate assimilation, unique fungal dissimilation mechanisms of nitrate that conserve energy under oxygen-limited conditions have been proposed. A denitrification mechanism was originally identified in *Fusarium oxysporum* during the 1990s (Shoun and Tanimoto 1991). This fungus reduces nitrate or nitrite to gaseous nitrous oxide under oxygen-limiting conditions via the activities of nitrate reductase (Nar), nitrite reductase (Nir), and NO reductase (Nor) (Fig. 9.1). Currently known filamentous fungi lack N_2O reducing activity, and the final product of fungal denitrification is not the N_2 that is generated by bacteria, but N_2O. The denitrifying fungus *F. oxysporum* produces Nar activity in mitochondria and in the cytosol, processes that are dependent on ubiquinol and NADPH, respectively (Fujii and Takaya 2008). Nitrate reduction by ubiquinol-Nar is associated with ADP phosphorylation, indicating the physiological significance of hypoxic denitrification as respiration (nitrate respiration). Genes encoding Nor have been isolated from *F. oxysporum*, *A. oryzae*, and other filamentous fungi, and they are essential for NO reduction to nitrous oxide (Takaya 2009). In contrast to bacterial Nor, which contains cytochrome *bc* as a cofactor, fungal Nor is unique among cytochrome P450 enzymes as it has an NO–heme complex heme in the active center to which NADH is a direct electron donor (Nakahara et al. 1993). The inducible production and ability of fungal Nor to oxidize NADH under hypoxic conditions suggest that it has a physiological role in dissimilating NO as an alternative electron acceptor to oxygen (Shoun and Tanimoto 1991; Fujii and Takaya 2008).

Another unique hypoxic mechanism in filamentous *F. oxysporum* and *A. nidulans* is ammonia fermentation (Zhou et al. 2002; Takasaki et al. 2004). These fungi reduce nitrate to ammonium, which is coupled with the oxidation of ethanol (carbon source) to acetate under hypoxic conditions, and ATP is then generated through

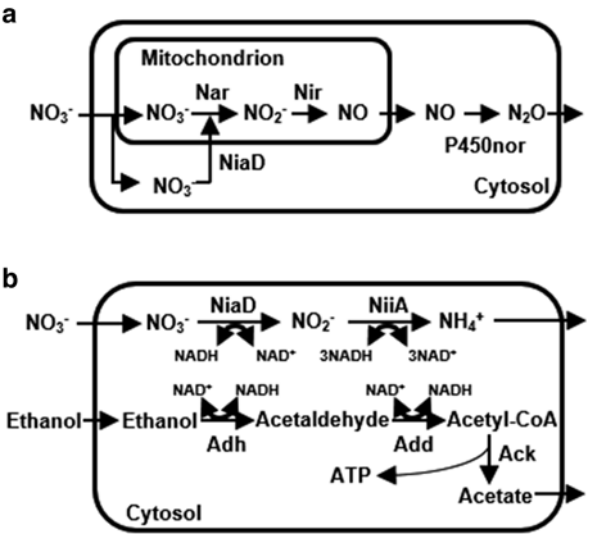

Fig. 9.1 Schematic representations of fungal hypoxic nitrogen metabolism. **a** Denitrification. **b** Ammonia fermentation. *Ack* acetate kinase, *Add* aldehyde dehydrogenase (acylating), *Adh* aldehyde dehydrogenase, *Nar* nitrate reductase, *NiaD and NiiA* Nar and Nir encoded by *niaD* and *niaD*, *Nir* nitrite reductase, *P450nor* cytochrome P450nor

substrate-level phosphorylation (Zhou et al. 2002; Takasaki et al. 2004). The enzymes involved in the oxidation of ethanol to acetate are alcohol dehydrogenase, coenzyme A-acylating aldehyde dehydrogenase, and acetate kinase (Ack). The key enzyme in this process is Ack, which generates acetate from acetyl-coA coupled with AMP and ADP phosphorylation in *A. nidulans* and *F. oxysporum*, respectively (Zhou et al. 2002; Takasaki et al. 2004). The nitrate-reducing reaction comprises nitrate and nitrite reductases (Takasaki et al. 2004), encoded by *niaD* and *niiA*, which are nitrate-assimilating enzymes in *A. nidulans*. These studies indicated that this fungus utilizes the nitrate-reducing mechanism to ammonium for both assimilation and dissimilation, the latter of which is a coping mechanism under low oxygen availability. The findings of denitrification and ammonia fermentation imply that fungal hypoxic mechanisms are far more complex than was previously thought.

9.2.2 Global Metabolic Changes Upon Hypoxia

Despite the potential significance of fungal hypoxic responses as dissimilation mechanisms, global metabolic changes remained obscure until recent genome-wide analysis using transcriptome and proteome approaches in the model fungus *A. nidulans* started to yield some clarification (Masuo et al. 2010; Shimizu et al. 2009). The transcriptional responses of *A. nidulans* in glucose minimal medium after transfer from normoxia to hypoxia were analyzed using DNA microarrays. The resulting transcriptome indicated that 27 % of the total number of genes was up- or downregulated by the altered oxygen availability (Masuo et al. 2010). The expression of genes for glycolysis, the tricarboxylic acid (TCA) cycle, and the

γ-aminobutyrate (GABA) shunt were upregulated upon hypoxia (Masuo et al. 2010). The same study found upregulated enzymes that convert pyruvate to ethanol and lactate under hypoxia, indicating that glucose in the medium is fermented to these compounds through glycolysis, and that this process generates ATP by substrate-level phosphorylation as it does in other microorganisms (ethanol and lactate fermentation) (Masuo et al. 2010). Glycolysis is also upregulated in *A. oryzae*, *A. fumigatus*, and *Saccharomyces cerevisiae* as well as in other filamentous fungi and yeasts (Terabayashi et al. 2012; Vödisch et al. 2011; Barker et al. 2012), implying that this phenomenon is conserved among fungi.

Transcripts associated with the TCA cycle are downregulated under hypoxic conditions in *Candida albicans*, *S. cerevisiae*, and *Trichoderma reesei*, whereas *A. niger* produces more intermediate metabolites of the TCA cycle under hypoxia, suggesting that TCA cycle metabolism is upregulated (Diano et al. 2009). Transcripts involved in the TCA cycle are induced, whereas the expression of respiratory genes is downregulated in *A. nidulans* (Masuo et al. 2010) as well as in *A. oryzae* (Terabayashi et al. 2012). Proteome studies of *A. nidulans* cells utilizing C-2 carbon (ethanol) showed that the production of TCA cycle enzymes does not change under hypoxia (Shimizu et al. 2009). The TCA cycle seems to be differently regulated in *A. fumigatus* when cultured under long-term hypoxia with limited glucose and under short-term hypoxia with sufficient glucose (Vödisch et al. 2011; Barker et al. 2012). These results suggest that culture conditions differentially regulate the TCA cycle among fungal species under hypoxic conditions. Detailed analyses are required to understand the regulation and physiological roles of the TCA cycle under hypoxia.

The GABA shunt requires glutamate dehydrogenase, glutamate decarboxylase, GABA transaminase, and succinic semialdehyde dehydrogenase; it bypasses two steps of the TCA cycle (Fig. 9.2). This pathway, which was discovered in plants around 50 years ago, is in fact conserved in almost all organisms (Bouché and Fromm 2004). The GABA and the GABA shunt are involved in various physiological processes such as neurotransmission, the regulation of carbon:nitrogen flux, and the oxidative stress response in diverse organisms, suggesting the critical importance of this pathway. Both transcripts and enzymes constituting this pathway are upregulated in *A. nidulans* under hypoxic conditions (Masuo et al. 2010; Shimizu et al. 2009). The GABA shunt is also upregulated in *A. oryzae*, *A. fumigatus*, and *F. oxysporum* (Terabayashi et al. 2012; Barker et al. 2012), implying that this bypass pathway is conserved among these filamentous fungi. Oxygen limitation under hypoxic conditions elevates the cellular NADH:NAD^+ ratio in *A. nidulans* (Masuo et al. 2010). The increased NADH level disrupts the cellular redox status, induces severe redox stress, and causes extreme metabolic changes in cells. Because the GABA shunt bypasses the NADH-generating step of TCA cycle, it is considered to be physiologically significant as a mechanism that prevents excessive NADH accumulation.

Hypoxia also causes changes in amino acid metabolism. Intracellular and extracellular alanine and glutamate levels are increased in *A. nidulans* under hypoxia

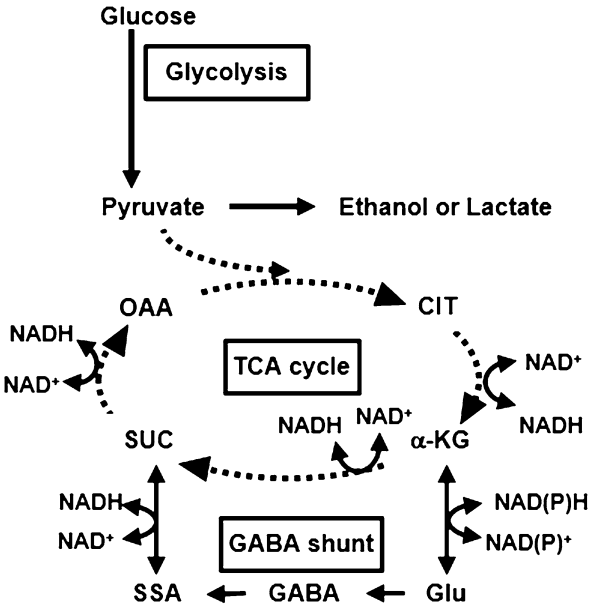

Fig. 9.2 Fungal hypoxic metabolism in glycolysis, tricarboxylic acid (TCA) cycle, and gamma-aminobutyric acid (GABA) shunt. *α-KG* α-ketoglutarate, *CIT* citrate, *GABA* γ-aminobutyrate, *Glu* glutamate, *OAA* oxaloacetate, *SSA* succinic semialdehyde, *SUC* succinate

(Masuo et al. 2010; Shimizu et al. 2010). These amino acids are the products of aminotransferase reactions against pyruvate and 2-oxoglutarate, as well as of upregulated glycolysis and the TCA cycle. Thus, levels of alanine and glutamate should increase under hypoxic conditions. Metabolism involving the GABA shunt is linked to the conversion of ammonium and pyruvate to alanine or glutamate, and it is likely to contribute to this phenomenon. *Aspergillus nidulans* overproduces branched-chain amino acids under hypoxia to regenerate NAD^+, which is referred to as branched-amino-acid fermentation (Shimizu et al. 2010). This process also seems to participate in the production of branched amino acids by hypoxic *A. fumigatus* cells, suggesting that these filamentous fungi maintain the intracellular NADH/NAD^+ balance via various mechanisms under hypoxic stress conditions.

Proteomic analysis has shown that hypoxic *A. nidulans* increases the production of enzymes associated with the pentose phosphate pathway (PPP) (Shimizu et al. 2009). One physiological role of PPP is the generation of NADPH, which serves as a substrate for the NADPH dehydrogenases that are involved in stress responses. Upregulated PPP might contribute to the ability of *A. nidulans* to tolerate redox states disrupted by hypoxia. The other role of PPP is the production of ribose-5-phosphate, which is a nucleotide precursor. *Aspergillus nidulans* cells accumulate more nitrite under hypoxia than in normoxia. Nitrite deaminates DNA purine bases under physiological conditions. Purine nucleotide metabolism is also activated in *A. nidulans* cells under hypoxia, indicating that this fungus repairs DNA damaged by accumulated nitrite under such conditions (Shimizu et al. 2009). Hypoxic organisms produce NO by reducing nitrite in mitochondria (Poyton et al. 2009). Although the mechanism of NO production by hypoxic fungal mitochondria remains

unclear, NO-detoxifying flavohemoglobin is upregulated in hypoxic *A. oryzae* and *A. fumigatus*, indicating a possible pathway (Terabayashi et al. 2012; Vödisch et al. 2011).

9.3 Nitric Oxide Responses of *Aspergillus nidulans*

9.3.1 Stress Imposed by Reactive Nitrogen Species and Responses of Filamentous Fungi

The findings of novel nitrate and RNS metabolic pathways in hypoxic *A. nidulans* cells opened up fungal RNS responses and adaptation as a new field of study. More knowledge of these pathways might be important to deepen understanding of the physiology of fermentative fungi because nitrate is often included in the fermentation media of filamentous fungi. Nitrite is an intermediate of nitrate reduction to ammonium, and it is in equilibrium with the powerful oxidant and mutagen, nitrous acid (HNO_2). Further protonation generates the highly reactive RNS nitrosonium (NO^+) (Poole 2005). Nitrate reductase (NADPH-dependent) in plants and probably isozymes in fungi produce NO as a by-product of nitrate reduction (Desikan et al. 2002). These nitrogenous oxides inhibit fungal activities under both hypoxic and aerobic conditions, and thus the fungal mechanisms that respond to and tolerate RNS stress should be considered to achieve maximal fermentation efficiency under both hypoxic and normoxic (aerobic) conditions.

Fungal mechanisms in response to RNS are best characterized as those to NO (Benhar et al. 2009). Upon exposure to exogenous NO, the yeast *Saccharomyces cerevisiae* induces the production of flavohemoglobin (Fhb) that oxygenates NO to nitrate and sequesters NO. Nitric oxide reacts with cellular thiols such as free cysteine, thiol moieties of antioxidant glutathione (GSH), and cysteine thiolate of proteins, and also generates *S*-nitrosothiols. The reaction with GSH generates *S*-nitrosoglutathione (GSNO), which GSNO reductase (GSNOR) reduces to GSH. The yeast GSH-GSNOR system and Fhb constitute a control mechanism of NO that eliminates stress imposed by RNS (Liu et al. 2001). The recent findings of Fhb in *A. oryzae* (Zhou et al. 2012) and *A. nidulans* (Zhou et al. 2013) indicate that these filamentous fungi have NO-detoxifying functions. However, the functions of the GSH/GSNOR system and other proteins in the responses of filamentous fungi to NO remain unknown.

9.3.2 Heme Biosynthesis

Zhou et al. screened *A. nidulans* for genes that tolerate NO to identify novel fungal mechanisms of RNS tolerance and generated 26,000 transformants from which six RNS-tolerant clones were isolated (Zhou et al. 2012). An *A. nidulans* genomic DNA

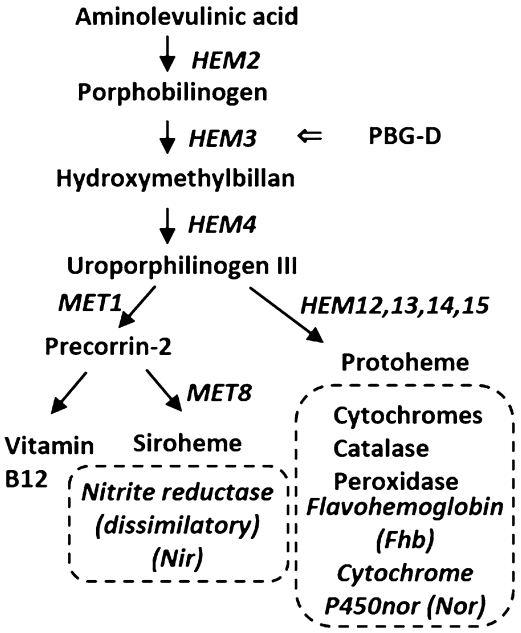

Fig. 9.3 Models of heme biosynthesis involved in reactive nitrogen species (RNS) tolerance. Heme biosynthetic pathway prediction of filamentous fungi. Corresponding *S. cerevisiae* genes are designated using conventional nomenclature

library along with a unique *A. nidulans–E. coli* shuttle vector (Gems et al. 1991) was introduced into *A. nidulans* and RNS-resistant transformants were then screened using acidified nitrite as the NO donor. This vector enabled recovery of the introduced plasmid by the simple transformation of *E. coli* with total DNA of the fungal transformants. As this type of vector cannot be used for other filamentous fungi, thus *A. nidulans* confers a strategic advantage when screening filamentous fungal genes for RNS tolerance.

Heme is an essential cofactor for the respiratory chain of the mitochondria and for some enzymes. The genetic screen for NO-tolerating genes previously described identified an orthologue of the *S. cerevisiae* porphobilinogen deaminase (PBG-D) gene (*HEM3*). This enzyme condenses four porphobilinogen molecules to form hydroxymethylbilane, which is an essential intermediate of the heme biosynthetic pathway (Fig. 9.3). The *A. nidulans* genome encodes a set of heme biosynthetic genes similar to those of yeast, indicating that this fungus synthesizes heme via the same metabolic pathway. A conditional mutant of the *HEM3* orthologue of *A. nidulans* (*hemC*) that expresses *hemC* under control of the inducible gene promoter confers a growth defect under the repressive conditions of *hemC* expression, indicating that this gene is critical for the normal growth of *A. nidulans* (Zhou et al. 2012). The growth of the mutant is more sensitive to acidified nitrite than the wild-type strain under *hemC*-repressive conditions. The cellular content of protoheme, which is found in most intracellular heme cofactors, decreases in *hemC*-repressed strains, indicating that the heme biosynthetic pathway is a novel determinant of cellular NO resistance.

Fungal enzymes that contain heme are involved in sterol synthesis, as well as sulfate and nitrate utilization. Adding sterol or other sulfur and nitrogen sources does not recover growth of the *hemC*-repressed strain that becomes defective in the presence of NO, indicating that the absence of this enzyme activity does not account for the NO-sensitive growth induced by a *hemC* deficiency. In fact, less Fhb-dependent NO dioxygenase activity is produced by the *hemC*-repressed strain than the wild-type strain. Intact cells of the strain consume NO more slowly than the wild type. These findings can be accounted for by *hemC* supplying protoheme to Fhb to upregulate NO detoxification (Fig. 9.3). The study of Zhou et al. was notable because it revealed more complex fungal RNS-detoxifying mechanisms; NADPH-nitrite reductase (NiiA, *niiA* gene product) reduces nitrite to ammonium, but detoxified acidified nitrite when added as the main NO donor under their experimental conditions. A contribution of PBG-D to growth in the presence of acidified nitrite has been suggested by adding an apoenzyme of NiiA with siroheme, which is an essential cofactor for producing enzyme activity.

9.3.3 Nitrosothionein

Another recently identified NO-tolerating gene of *A. nidulans* encodes a 23-amino-acid peptide called inducible nitrosothionein (iNT) (Zhou et al. 2013). This peptide is similar to the N-terminal half of metallothionein (MT) (Fig. 9.4), which is a ubiquitous, cysteine-rich peptide constituting N- and C-terminal β- and α-domains that both contain several cysteine resides (Capdevila and Atrian 2011). The overproduction of iNT or its loss induced by gene disruption respectively increases or decreases fungal growth in the presence of acidified nitrite as the NO donor, indicating that iNT is involved in the NO tolerance mechanism of *A. nidulans*. The gene disruptant of the gene encoding iNT produces less aconitase activity, and the fungus becomes more susceptible to respiratory oxygen consumption than the wild type after exposure to acidified nitrite. These impairments are likely to be involved in the retarded growth of the iNT gene disruptant because these activities are essential cellular metabolic processes.

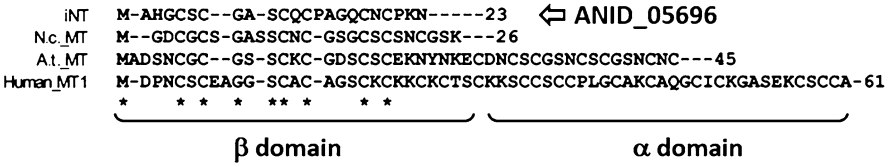

Fig. 9.4 Nitrosothionein and RNS tolerance. Alignment of nitrosothionein (iNT), classical metallothioneins, and fungal iNT-like peptide. Cysteine residues are *highlighted. A.t. Arabidopsis thaliana*, *N.c. Neurospora crassa*

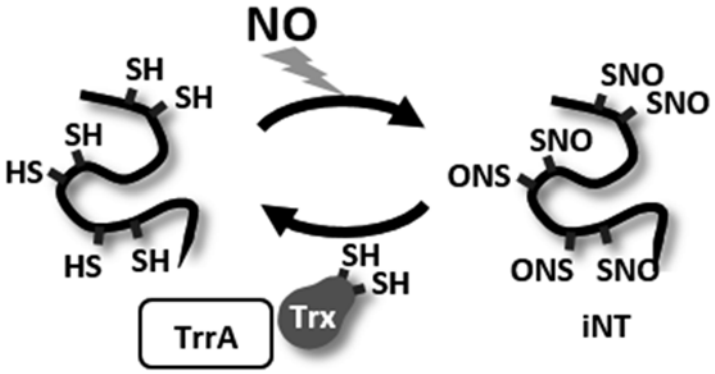

Fig. 9.5 Role of iNT coupled with thioredoxin (*Trx*) system in NO detoxification. *TrrA* thioredoxin reductase encoded by *trrA*

The chemistry of thiols (–SH) and NO is established, and their reactions under physiological conditions generates *S*-nitrosothiols (–SNO). This is also true for the iNT peptide, and thiols of the six cysteine residues are stoichemetrically converted to *S*-nitrosothiols in vitro (Zhou et al. 2013). Studies using the gene disruptant and a strain that produces an excess of iNT found a negative correlation between cellular iNT levels and the amount of *S*-nitrosated proteins, indicating that iNT protects cellular protein thiols from *S*-nitrosation by scavenging NO under RNS stress. The NO scavenging role of iNT seems to be mediated by thioredoxin-dependent catalysis (Zhou et al. 2013). Reconstitution studies in vitro have shown that thioredoxin (TrxA) rapidly reduces *S*-nitrosated iNT (iNT-SNO) to iNT, and the resultant oxidized thioredoxin is reduced by thioredoxin reductase (TrrA) and NADPH (Fig. 9.5). Reverse genetics studies have demonstrated that the NO-tolerance function of iNT requires TrrA, and the evidence described here supports this conclusion.

Metallothionein binds heavy metal cations at both the α- and β-domains, decreases their cellular concentration, and thus induces tolerance against them. Judging from its structural similarity to the metallothionein β-domain, the iNT peptide not only reacts with NO but also efficiently binds copper (I) (Zhou et al. 2013), suggesting that metallothionein and iNT both bind metals. However, disruption of the iNT gene does not affect fungal growth in the presence of various heavy metals. This is evident in the genetic background of this fungus that lacks typical metallothioneins, suggesting that iNT is dispensable for fungal heavy metal tolerance in vivo. This discrepancy between the chemical and physiological functions of iNT in metal tolerance might be explained by the regulation of iNT production. Zhou et al. found that adding acidified nitrite increases iNT gene expression, and that intracellular levels of iNT consequently increase to 5.0 ± 1.0 nmol g protein^{-1}, which is comparable to that of metallothionein in other organisms. This finding indicates that under normal conditions without added NO, the amount of iNT is insufficient to cause heavy metal tolerance, although this remains to be confirmed.

The physiological roles of *S*-nitrosothiols have recently been investigated in detail and iNT has provided a novel example of RNS-tolerant peptides in addition to GSH. In contrast to the metal-binding ability of iNT, thiol apparently imposes *S*-nitrosothiol generation on metallothionein cysteine residues both in vitro and in vivo. Mammalian metallothionein-3 is not involved in heavy metal tolerance (Vašák and Meloni 2011). Although NO-related functions remain unknown, NO induces the gene expression of metallothionein in mesangial cells (Datta and Lianos 2006). These metallothioneins might constitute an NO-detoxifying machinery similar to that of iNT.

9.4 Future Prospects

The metabolic mechanisms of *S. cerevisiae* under hypoxia and NO have been characterized in detail and those of filamentous fungi are just beginning to emerge. Both *S. cerevisiae* and *Aspergillus* are grouped in the phylum Ascomycotina or as imperfect fungi that are closely related to Ascomycotina. Their evolutional relationship indicates that they share some of the same response mechanisms to exogenous stimuli, such as upregulated glycolysis in response to hypoxia and the involvement of Fhb in NO tolerance. However, *S. cerevisiae* is unequivocally different in that it grows under hypoxic (and probably under anoxic) conditions, implying that *Aspergillus* will supersede this yeast under hypoxia as the most important model organism for studying general fungal responses to hypoxia. Furthermore, the fact that filamentous fungi produce proteins related to nitrogen oxides and RNS metabolism that have never been found in *S. cerevisiae* is hardly surprising because filamentous fungi efficiently reduce nitrate for utilization as a nitrogen source and electron acceptor. These proteins include cytochrome P450nor, iNT, and nitrate/nitrite reductases, as described in this chapter. Other proteins are being discovered in filamentous fungi. Genes regulating hypoxic gene expression and RNS regulation in filamentous fungi as well as cues that could explain the overall scheme of responses will be discovered in the near future.

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