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Surviving the odds: from perception to survival of fungal phytopathogens under host-generated oxidative burst

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ABSTRACT

Fungal phytopathogens pose a serious threat to global crop production. Only a handful of strategies are available to combat these fungal infections, and the increasing incidence of fungicide resistance is making the situation worse. Hence, the molecular understanding of plant–fungus interactions remains a primary focus of plant pathology. One of the hallmarks of host–pathogen interactions is the overproduction of reactive oxygen species (ROS) as a plant defense mechanism, collectively termed the oxidative burst. In general, high accumulation of ROS restricts the growth of pathogenic organisms by causing localized cell death around the site of infection. To survive the oxidative burst and achieve successful host colonization, fungal phytopathogens employ intricate mechanisms for ROS perception, ROS neutralization, and protection from ROS-mediated damage. Together, these countermeasures maintain the physiological redox homeostasis that is essential for cell viability. In addition to intracellular antioxidant systems, phytopathogenic fungi also deploy interesting effector-mediated mechanisms for extracellular ROS modulation. This aspect of plant–pathogen interactions is significantly under-studied and provides enormous scope for future research. These adaptive responses, broadly categorized into "escape" and "exploitation" mechanisms, are poorly understood. In this review, we discuss the oxidative stress response of filamentous fungi, their perception signaling, and recent insights that provide a comprehensive understanding of the distinct survival mechanisms of fungal pathogens in response to the host-generated oxidative burst.

Key words: reactive oxygen species (ROS), oxidative stress response, fungal effectors, stress signaling, plant–pathogen interactions

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INTRODUCTION

In contrast to animals, plants—being immobile—encounter a wide array of opportunistic micro- and macroorganisms that exert positive or negative effects on overall plant health and crop yield (Segal and Wilson, 2018). Modern monoculture-based agriculture suffers huge crop losses due to invading pathogens, among which fungi are of immense economic importance. Diverse pathogenic lifestyles such as biotrophy, hemi-biotrophy, and necrotrophy (Lo Presti et al., 2015) are currently thought to be different phases of infection and disease progression (Lorang, 2019). The two-tiered plant immune system spontaneously responds to pathogen-associated molecular patterns (PAMPs) recognized from the invading fungal pathogen. This triggers the first level of defenses, such as the oxidative burst, callose deposition, and increased pathogenesis-related (PR) gene expression, cumulatively referred to as PAMP-triggered immunity (PTI). To manipulate PTI responses, fungal pathogens send a second wave of attack composed of secreted effector proteins. The recognition of effectors by the host may lead to either effector-triggered susceptibility (ETS) or an aggressive form of PTI referred to as effector-triggered immunity (ETI) (Jones and Dangl, 2006). The outcome of effector recognition determines the winner of the war: ETS indicates defeat for the plant host and ETI suggests victory. However, the current understanding of plant immunity suggests that certain

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PAMPs are narrowly conserved among the filamentous phytopathogens and that their functions overlap with those of virulence effectors, and vice versa. These aspects blur the boundary between the dichotomy of PTI and ETI (Thomma et al., 2011; van der Burgh and Joosten, 2019).

The host-produced oxidative burst, marked by the production of radical and non-radical reactive oxygen species (ROS) such as singlet oxygen, superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl ion, is considered to be a generalized host defense against pathogen attack in both plants and animals (Klotz, 2002; Heller and Tudzynski, 2011). ROS, typically produced by enzymes such as the NADPH oxidases (Nox) or so-called "leaky" mitochondria (Breitenbach et al., 2015), can be extremely harmful and may directly damage DNA, RNA, polysaccharides, lipids, proteins, and smaller metabolites (Beckman and Ames, 1998; (Heller and Tudzynski, 2011). To maintain redox equilibrium, oxidative stress defense systems often employ peroxiredoxins to reduce H_2O_2, nitric oxide (NO), and alkyl hydroperoxides using reducing equivalents provided by NADPH ((Hall et al., 2009); Nelson et al., 2011; (Nelson and Parsonage, 2011); Stincone et al., 2014). In addition to peroxiredoxins, fungal phytopathogens possess other antioxidant systems such as catalases and glutathione peroxidases (Stincone et al., 2014; Breitenbach et al., 2015). A recent study shows that metabolic enzymes, putative effectors, and other virulence-related proteins are induced in the necrotrophic fungus *Ascochyta rabiei* under oxidative stress (Maurya et al., 2020). This indicates a possible correlation between fungal stress response and pathogenicity, although how fungal pathogens colonize the host plant despite the deadly oxidative burst still remains poorly understood. Hence, the only logical argument is that during the course of evolution with the host, fungal phytopathogens have acquired novel ways to combat host-generated oxidative stress and have exploited this hostility for their own benefit.

In this review, we discuss the unique oxidative stress responses of the filamentous fungal phytopathogens and the means by which these defense responses assist them in coping with the host-generated oxidative burst. Furthermore, we explore the biological significance of ROS for fungal growth and development during the plant defense response with regard to disease establishment, and we highlight the molecular basis of coordinated fungal defense mechanisms. Finally, we summarize the current understanding of these fungal phytopathogen survival strategies and provide insights into future research implications for the control of fungal diseases in crops.

THE BIRTH, BOON, AND BANE

Production of reactive oxygen species in the host and pathogen

The aerobic lifestyle constantly generates ROS as by-products of active metabolic processes that occur in different subcellular compartments such as chloroplasts, peroxisomes, and mitochondria. Incomplete reduction of molecular oxygen during energy or electron transfer reactions leads to ROS production (Singh et al., 2016b). The host plant produces ROS as a defense mechanism under various abiotic and biotic stresses to cope with the hostile environment, whereas fungi require ROS for their own developmental processes (Figure 1). In the apoplast, ROS are produced by cell-wall peroxidases and the membrane-localized Nox complex, which are members of the "respiratory burst oxidase homologs" family (Kadota et al., 2015; Scott, 2015). The fungal Nox complex has been extensively studied in *Botrytis cinerea*, *Magnaporthe oryzae*, and *Podosphaera anserina* (Lacaze et al., 2015; Siegmund et al., 2015; Galhano et al., 2017). A NoxD mutant of *B. cinerea* shows growth defects under high ROS conditions, suggesting that the NoxD adapter protein has an indispensable role in proper Nox function. NoxC possesses a calcium-binding EF hand motif and is similar to mammalian homolog Nox5 (Segal and Wilson, 2018). Moreover, the transient overexpression of protein disulfide isomerases, abundant redox proteins of endoplasmic reticulum origin, promotes Nox activation and ROS production (Laurindo et al., 2012). In addition to Nox, glucose oxidases, a group of flavin- and iron- or copper-containing enzymes, can reduce dioxygen to H_2O_2. Xanthine oxidases and lipoxygenases are other enzymes that also generate ROS (Mayer et al., 2001).

As discussed earlier, pathogens elicit several basal immune responses in plants, including ROS accumulation. The citrus-infecting necrotrophic fungus *Alternaria alternata* triggers lipid peroxidation and H_2O_2 accumulation during host colonization. The rough lemon pathotypes are capable of producing a host-selective ACRL toxin that perturbs mitochondrial function and RNA splicing, resulting in abnormal oxidative phosphorylation and metabolite leakage in the susceptible host (Chung, 2012). Similarly, *Ganoderma boninense*-inoculated roots of the oil palm *Elaeis guineensis* enhance ROS production through salicylic acid (SA) accumulation; this confers resistance against hemibiotrophs but increases susceptibility to necrotrophs. The SA-mediated signaling negatively regulates the expression of ascorbate oxidase and ascorbate peroxidase, which function directly in ROS scavenging (Ho et al., 2016). Moreover, during infection, plants secrete a large number of enzymes into the apoplast to combat fungal invasion. Interestingly, maize roots inoculated with the opportunistic root endophyte *Trichoderma virens* showed a 50% reduction in host-secreted peroxidases, and enzymes such as superoxide dismutase, glutathione *S*-transferase, peroxiredoxin, and thioredoxins were not present at all, whereas the reverse was the case in uninoculated roots (Nogueira-Lopez et al., 2018). Although the utilization of host-generated ROS is a feature of necrotrophs, in this case a mutualistic endophyte suppresses the host's antioxidant activity to maintain the ROS levels required for the establishment of beneficial associations.

ROS: a critical factor for fungal life

In addition to their deleterious effects, ROS and reactive nitrogen species (RNS) are also essential for the regulation of signal transduction pathways. Pathogen-induced ROS and RNS have emerged as key players in pathogenesis-related and developmental processes of filamentous fungi (Figure 1). The equilibrium between ROS production by the host and stress response by the phytopathogenic fungi is the axis of host–pathogen interactions. The animal pathogenic fungus *Candida albicans* encodes an NADPH oxidase, Fre8, which together with

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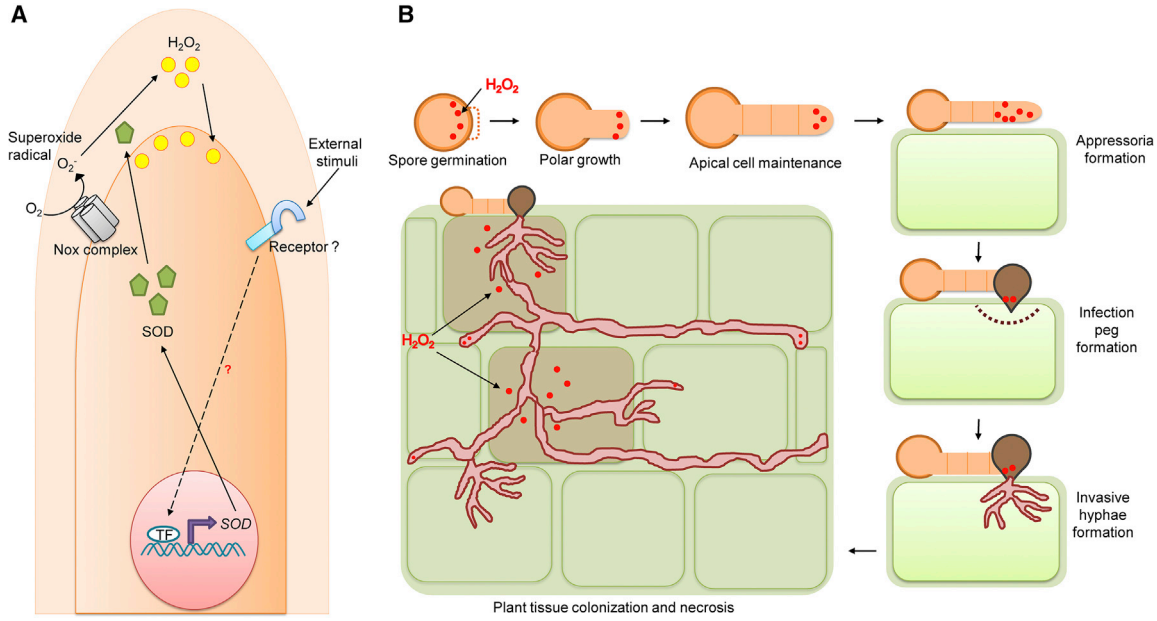

Figure 1. Reactive oxygen species mediate fungal growth and pathogenesis.

(A) Filamentous fungi modulate hyphal growth in response to biotic and abiotic stresses. The perception of external stimuli by unknown membrane-bound or cytoplasmic receptors transcriptionally activates defense-related genes such as *SOD*. In the extracellular space of the apical cell, membrane-bound fungal Nox complexes constantly generate superoxide radicals from oxygen molecules as part of their physiological function. Superoxide radicals are neutralized by extracellular SOD to form H_2O_2 at the growing hyphal tip. H_2O_2 acts as a chemical trigger for cell wall integrity and remodeling processes and regulates directional growth in filamentous fungi.

(B) Fungal spore germination requires oxidative stress, particularly H_2O_2 localization at the site of germ-tube initiation, and controls polar growth and germ-tube length. It also mediates apical dominance and fruiting body development during vegetative growth. During plant–fungus interactions, ROS regulate fungal cell differentiation and the formation of specialized infection structures such as appressoria, the infection peg, and invasive hyphae. ROS negatively affect cell wall permeability and integrity, and assist the fungus in penetrating the host cell wall. Once inside the host, necrotrophic fungi secrete large amounts of ROS to kill the host in order to derive nutrition and proliferate within the plant.

Sod5 generates a H_2O_2 gradient outside the cell to support hyphal growth (Rossi et al., 2017). Similarly, H_2O_2 acts as a chemical signal for chemotropic growth of *Fusarium oxysporum* toward a host-secreted peroxidase gradient (Nordzieke et al., 2019) (Figure 1A). Mild oxidative stress has also been shown to improve polarized growth under *in vitro* conditions in a thioredoxin-dependent manner (Nasution et al., 2008; da Silva Dantas et al., 2010). During *B. cinerea* infection, O_2^- accumulates in the fungal hyphal tips, and H_2O_2 is generated through Nox complexes around the host cell wall and plasma membrane (Figure 1B). Loss of the Nox complex results in delayed appressoria development and altered morphology (Tenberge et al., 2002; Egan et al., 2007; Segmüller et al., 2008). Substantial evidence suggests that ROS are involved in fungal differentiation processes. For example, *Aspergillus nidulans* requires Nox-produced ROS for fruiting body development and apical dominance (Lara-Ortíz et al., 2003; Semighini and Harris, 2008). In *Sclerotium rolfsii*, the biogenesis and differentiation of sclerotia, which are asexually propagating structures, are characterized by high lipid peroxidation and oxidative stress caused by free radicals (Georgiou et al., 2003). Moreover, the deletion of the flavohemoglobin gene (FhbA), which encodes a conserved protein that reduces nitric oxide (NO), induces sexual development and decreases sterigmatocystin production in *A. nidulans*. These findings indicate that NO plays a role in fungal morphogenesis and secondary metabolism (Baidya et al., 2011). Recently, uredinial germination in the wheat stripe rust pathogen *Puccinia striiformis* Westend. f. sp. *tritici* has been found to be regulated by NO and ROS, and scavengers of ROS and NO delay spore germination and reduce germ-tube length. Furthermore, the study suggests that a balanced ROS/NO ratio is crucial for normal polar growth of the germ tube (Yin et al., 2016). The targets of ROS and their precise role in fungal development are still unclear. However, the findings discussed above imply a critical role for ROS and RNS in the regulation of various fungal differentiation processes ranging from spore germination to the formation of sexual and infection structures, i.e., penetration hyphae, appressoria, and so forth (Figure 1).

The wicked side of ROS

Under normal physiological conditions, intracellular ROS molecules are readily neutralized by the antioxidant defense system. However, under adverse conditions such as plant defense responses, the balance between production and scavenging is perturbed. This causes an increase in cellular ROS level that can be fatal for the structural and functional integrity of an array of biomolecules. Therefore, prolonged exposure to ROS can cause cell death (Chung, 2012) (Figure 2). For example, superoxide ions can damage the cell membrane through oxygen toxicity and photo-oxidation (Mayer et al., 2001). Superoxide radicals have a very short half-life but are also very reactive, unlike H_2O_2, which can move across the cell membrane. ROS can

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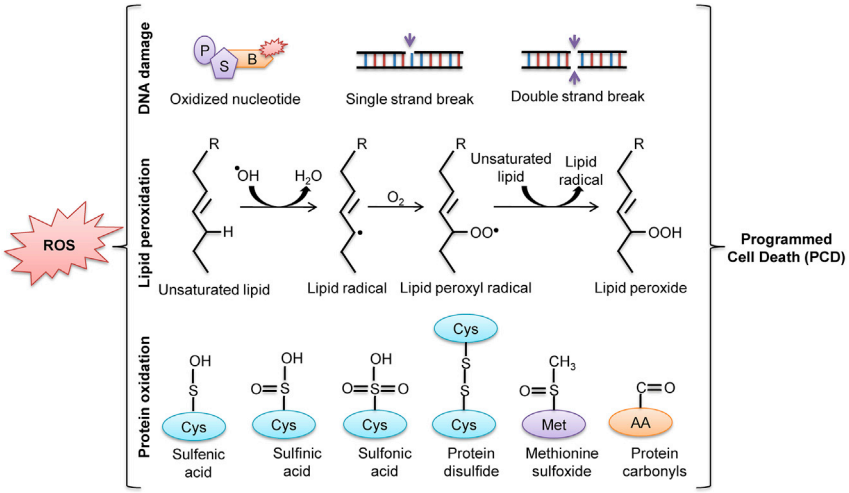

P, Phosphate; S, Deoxyribose sugar; B, Nitrogenous base; Cys, Cysteine; Met, Methionine; AA, Amino acid

Figure 2. ROS-mediated oxidative modifications of biological macromolecules.
Elevated ROS levels negatively affect pleiotropic cellular processes through their damaging effects on important biomolecules such as DNA, lipids, and proteins. They can non-specifically oxidize purine and pyrimidine nucleotides, which can cause single- or double-stranded breaks in DNA and can give rise to genomic instability. Another deleterious effect of ROS, lipid peroxidation is initiated by the hydroxyl radical, leading to the formation of the lipid radical, which further forms the lipid peroxyl radical in the presence of oxygen. The lipid peroxyl radical combines with another unsaturated lipid to again form the lipid radical and lipid peroxide. In addition, ROS cause reversible or irreversible oxidative modifications in polypeptides and proteins, including the formation of sulfenic acid, sulfinic acid, sulfonic acid, and protein disulfide involving redox-sensitive cysteine thiols. Methionine residues can also be oxidized to methionine sulfoxide, and under extreme oxidative stress, irreversible protein carbonylation takes place. All these oxidative modifications perturb the normal physiological function of proteins involved in critical cellular processes. Taken together, irreparable ROS-mediated oxidative damage can induce PCD.

oxidize many biological macromolecules such as lipids and proteins in a non-specific manner, resulting in their loss of function or abnormal behavior (Figure 2). The oxidative mutagenesis of DNA includes base modifications, point mutations, single-strand breaks, intra- and inter-strand DNA crosslink formation, and lesions that can block replication and result in double-strand breaks. All these are reasons for the genomic instability that gives rise to tumors and cancers (Tsang et al., 2014) (Figure 2). ROS-mediated oxidative modifications occur spontaneously at cysteine, methionine, histidine, and tyrosine residues in polypeptides (Figure 2). These modifications can be reversible (e.g., glutathionylation and disulfide crosslinking) or irreversible (oxidations such as protein carbonylation) (Ezraty et al., 2017). Recently, the significance of such small oxidative modifications in actin protein has been discovered: methionine (Met^{44}, Met^{47}) oxidation by the flavin mono-oxygenase enzyme MICAL1 results in rapid actin depolymerization and disrupts normal actin dynamics in *Drosophila* (Hung et al., 2011; Grintsevich et al., 2017). Therefore, ROS and RNS molecules together have the potential to cause catastrophic effects, leading to cell arrest in a range of organisms that lack suitable defense machinery.

PERCEPTION AND SIGNAL TRANSDUCTION DURING OXIDATIVE STRESS: A COMPLEX INTERPLAY

Mitogen-activated protein kinase signaling

In eukaryotes, the mitogen-activated protein kinase (MAPK) phosphorylation relay is the central component of adaptation signaling in response to various stress stimuli. Histidine kinase (bos1) and MAPK (sak1) participate in signal transduction pathways for fungal oxidative stress response (Liu et al., 2008) (Figure 3). In *B. cinerea*, mutants of *bos1* and *sak1* have low levels of mannitol dehydrogenase, BcSOD, CAT7, BcTRX, PRX1, and PRX9, which are necessary for intracellular ROS neutralization (Kilani et al., 2020). Upon silencing of the stress-associated MAPK PsMPK7, *Phytophthora sojae* exhibits increased sensitivity to H_2O_2 and accumulates a higher level ROS at the infection site, similar to *Δvdpbs2* mutants of *Verticillium dahliae* (Gao et al., 2015; Tian et al., 2016) (Figure 3). In *Bipolaris oryzae*, SRM1 (a homolog of Hog-1 type MAPK) controls the expression of the catalase (*CAT2*) gene and confers tolerance to oxidative stress and other abiotic stresses (Moriwaki et al., 2006). In *Fusarium verticillioides*, lack of FvBck1, an MEK homolog critical for stress response and virulence, impedes the activity of ROS-scavenging enzymes such as peroxidases, superoxide dismutases, ascorbate oxidases, and catalases and also hampers the production of the mycotoxin fumonisin B1 (Zhang et al., 2015) (Figure 3). More recently, the fungal MAPK Pmk1 has been shown to be required for cell-to-cell invasion and proliferation of *M. oryzae* in a rice host. Furthermore, Pmk1 regulates the transcript-level expression of secreted effector proteins such as BAS1 and Avr-Pita, which are implicated in the suppression of host immunity, ROS generation, and callose deposition at plasmodesmata (Sakulkoo et al., 2018). Together, these findings highlight the extensive involvement of MAPK signaling components in oxidative stress adaptation during host colonization by phytopathogenic fungi (Figure 3).

ROS-mediated signaling

In plants, the recognition of ROS during plant–pathogen interaction is known to occur in the apoplast (Buron-Moles et al., 2015). Recently, a breakthrough report identified a plasma membrane-localized H_2O_2 receptor named hydrogen-peroxide-induced Ca^{2+} increases (HPCA1), which is a leucine-rich receptor kinase in *Arabidopsis*. HPCA1 is activated via H_2O_2-mediated covalent modification of extracellular cysteines; H_2O_2 is then transported inside the host cell through aquaporins, causing the cell to mount defense responses (Wu et al., 2020). In fungi, no such membrane-bound ROS receptors have been identified to date, although a few cytosolic ROS sensor proteins are well documented.

One of the most studied examples is yeast-activating protein 1-like (YAP1), which acts as a transcriptional regulator of oxidative

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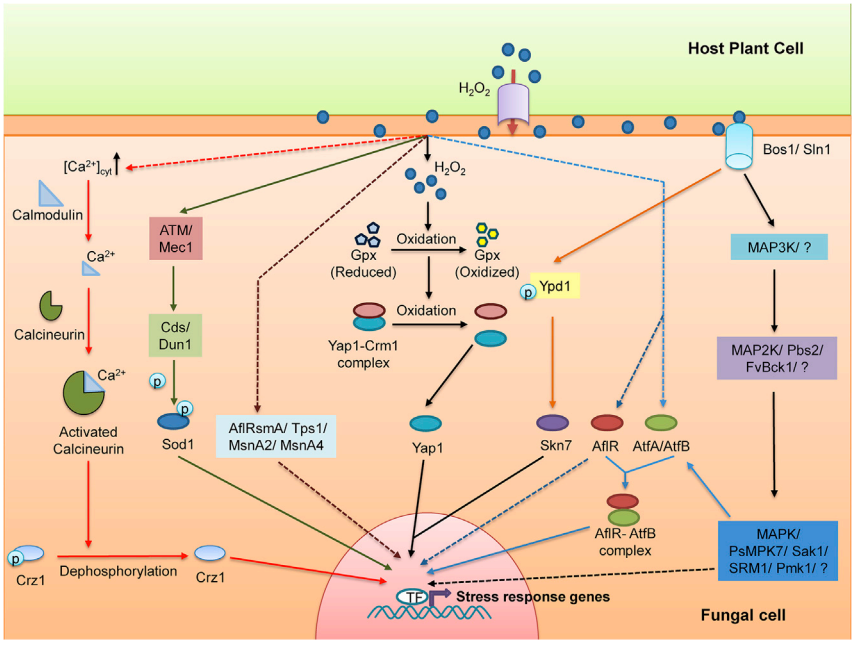

Figure 3. Oxidative stress signaling in phytopathogenic fungi.

Extracellular ROS increase $[Ca^{2+}]_{cyt}$ signatures, which act as secondary messengers in signaling cascades. Ca^{2+} binds to calmodulin, followed by calcineurin activation via the Ca^{2+}-calmodulin-calcineurin pathway. Calcineurin-mediated dephosphorylation triggers nuclear localization of the Crz1 transcription factor for transcriptional activation of stress response and cell wall integrity genes. In the cytosol, freely diffusing H_2O_2 is perceived by the redox-sensitive thiols of glutathione peroxidases (Gpx), which oxidize cysteine thiols of Yap1 in a redox cascade and disrupt the Yap1–Crm1 complex. Oxidized Yap1 translocates to the nucleus and transcriptionally regulates antioxidant genes. Other transcription factors such as AflRsmA, Tps1, and MsnA2/MsnA4 are also involved in oxidative stress tolerance; however, their upstream activation mechanisms remain unclear. The ATM/Mec1-mediated activation of Cds/Dun1 kinase leads to phosphorylation-triggered nuclear localization of SOD1, which prevents DNA damage and regulates catalase gene expression. A histidine sensor kinase (Bos1/Sln1) phosphorylates Ypd1, which activates Skn7 in a phosphorelay to work with Yap1 against oxidative stress. In parallel, Bos1 initiates a MAPK phosphorelay involving MAP2Ks such as VdPbs2 and FvBck1, as well as PsMPK7, Sak1, SRM1 (a Hog1 homolog), and Pmk1, which are essential for antioxidation and for the regulation of aflatoxin biosynthesis-related transcription factors AtfA/AtfB. Coordinated activation of these signaling cascades orchestrates oxidative stress defense response in fungi.

stress-responsive and antioxidant genes in *Saccharomyces cerevisiae*. Yap1 is a basic leucine zipper (bZIP) transcription factor that preferentially activates glutathione and thioredoxin systems (Hong et al., 2013). Under normal conditions, the nuclear export protein Crm1 recognizes a conserved nuclear export signal in the cysteine-rich domain of Yap1 and prevents its nuclear retention. During oxidative stress, however, H_2O_2 oxidizes glutathione peroxidase 3 (GPx3), which subsequently oxidizes the cysteine residues of Yap1 (Figure 3). This produces a redox conformational change that causes Yap1 to dissociate from Crm1, translocate to the nucleus, and promote the transcription of antioxidant genes (Kuge et al., 1998; Toone et al., 1998; Delaunay et al., 2002) (Figure 3).

Many Yap1 homologs have been studied in plant–pathogen interactions, but their functional significance in biotrophic and mutualistic fungi is still unclear. For example, the Yap1 homolog of the biotrophic maize pathogen *Ustilago maydis* (UmYap1) localizes to the nucleus upon H_2O_2 exposure, and its mutants are avirulent. This suggests that *U. maydis* depends on ROS quenching, as well as other roles for UmYap1 (Molina and Kahmann, 2007). The MoAP1 and MoTRX2 mutants of *M. oryzae* accumulate more ROS and show a reduced pathogenicity phenotype (Kou et al., 2019). Yap1 homologs of *Aspergillus parasiticus* and *A. alternata* are fundamental to fungal virulence, however, it has a minor role in the virulence of *Cochliobolus heterostrophus* (Lev et al., 2005; Lin et al., 2009; Chung, 2012; Reverberi et al., 2012; Hong et al., 2013). Similarly, Pap1 in *Schizosaccharomyces pombe*, Cap1 in *C. albicans*, and Kap1 in *Kluyveromyces lactis* promote the transcription of *CTT1*, *TRX2*, *TRR1*, *SOD1*, and other genes (Hong et al., 2013). In contrast to their critical role during plant–fungus interactions, Yap1 homologs are sometimes dispensable for fungal virulence. In the case of *B. cinerea*, BAP1 is necessary for neutralizing ROS in axenic culture but not during plant invasion (Temme and Tudzynski, 2009). Interestingly, in *Fusarium graminearum*, FgAp1 mediates the oxidative stress response, which activates *TRI* genes for the production of mycotoxins and other secondary metabolites of the trichothecene family (Arunachalam and Doohan, 2013; Montibus et al., 2013).

In addition to YAP1, Skn7 is another major transcription factor and is activated by the phosphotransferase Ypd1 under oxidative stress in *Saccharomyces* spp. Skn7 drives the expression of many antioxidant genes such as those encoding catalase, superoxide dismutase, and thioredoxin (Jiang et al., 2015) (Figure 3). The deletion mutants of *aflSkn7* and *fgskn7* are unable to perform H_2O_2-induced *TRI* gene expression and show extreme sensitivity to ROS (Jiang et al., 2015; Zhang et al., 2016a). More recently, the Δ*mrskn7* mutants of *Monascus ruber* were also found to be highly sensitive to peroxides (Shao et al., 2017). Deletion of the bZIP transcription factor AtfA, MAPK, stress-activated kinase A (SakA), and catalase A (CatA) renders the conidia of *Aspergillus* spp. hypersensitive to H_2O_2 (Sakamoto et al., 2009; Lara-Rojas et al., 2011). Consistently, the *moatf1* mutant of *M. oryzae* shows increased sensitivity to ROS and reduced virulence (Jiang et al., 2015). In *B. cinerea*, BcAtf1 controls catalase B expression but is not entirely involved in the oxidative stress response, whereas the *cptf1* mutant of *Claviceps purpurea* elicits a host ROS response that inhibits its own growth (Nathues et al., 2004; Temme et al., 2012; Hong et al., 2013). Two other transcription factors, AflRsmA and AtfB, also coordinate antioxidant gene expression (Wang et al., 2020) and aflatoxin biosynthesis (Hong et al., 2013; Wee et al., 2017) (Figure 3). Extracellular ROS signals modulate the activity of AflR, which positively regulates

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aflatoxin synthesis in *Aspergillus* spp. In a MAPK cascade, the AtfB–AflR complex transcriptionally regulates aflatoxin synthesis, antioxidant and developmental genes (Roze et al., 2015) (Figure 3).

In addition, the glucose-6-phosphate/NADPH-sensing enzyme Tps1 also regulates the transcription of NADPH-dependent antioxidant genes, which are part of the glutathione and thioredoxin systems in *M. oryzae* (Fernandez and Wilson, 2014). The Cys2His2 zinc finger transcription factors MsnA2 and MsnA4 were upregulated during oxidative stress, heat shock, and starvation, and activated the expression of *CTT1* in yeast (Martinez-Pastor et al., 1996; Hong et al., 2013). Interestingly, the deletion of *Sod1* in yeast and mice can expose the cell to DNA damage by ROS. Elevated ROS levels activate the oxidative stress sensor Mec1/ATM kinase, which ultimately leads to the Dun1/Cds1 kinase-mediated phosphorylation of Sod1 (Figure 3). Phosphorylated Sod1 rapidly localizes to the nucleus and binds to the promoters of oxidative stress resistance and repair genes to maintain genomic stability (Tsang et al., 2014). Moreover, a mutation in the yeast elongator complex 3 (ELP3) ortholog made *F. graminearum* defective in trichothecene production, less virulent, and hypersensitive to oxidative stress as catalase expression was suppressed (Lee et al., 2014).

In *Trichoderma atroviride* and *Aspergillus awamori*, mechanical injuries evoke transient oxidative responses such as the activation of calcium signaling pathways and oxylipin production (Nelson et al., 2004; Hernández-Oñate et al., 2012). Ca^{2+}/calcineurin signaling, one of the major stress response pathways, is widely conserved in pathogenic fungi (Liu et al., 2015a; Muñoz et al., 2015). The deletion of Ca^{2+}/calmodulin-dependent serine-threonine kinase 2 (cmk2) increases the sensitivity of *S. pombe* to oxidative stress (Sanchez-Piris et al., 2002; Muñoz et al., 2015; Tisti et al., 2016). Calcineurin, a Ca^{2+}/calmodulin-dependent serine-threonine phosphatase, is essential for fungal growth inside the host, as well as for oxidative and osmotic stress tolerance of *Cryptococcus neoformans* (Park et al., 2019). The cytosolic phosphoprotein calcineurin responsive zinc finger 1 (Crz1) transcription factor is one of the substrates of calcineurin and acts as a major stress response regulator. During stress, the cytosolic Ca^{2+} spike activates calcineurin, which in turn dephosphorylates Crz1, resulting in its rapid nuclear localization to regulate target gene expression (Chow et al., 2017) (Figure 3). Crz1 homologs activate cell wall remodeling genes under stress conditions and mediate appressorium formation, host penetration, and virulence of *M. oryzae* and *Magnaporthe grisea* (Choi et al., 2008; Zhang et al., 2009). $CaCl_2$ stimulates ROS production through its interference with BcNoxA and BcNoxB in *B. cinerea* (Marshall et al., 2016a, 2016b). The *Δbccrz1* mutants of *B. cinerea* are defective in hyphal morphology, conidiation, and sclerotium formation under alkaline, calcium, lithium, and oxidative stresses (Schumacher et al., 2008). Disruption of PdCrz1 in *Penicillium digitatum* reduces conidiation by 90% and makes the fungus sensitive to Ca^{2+} and oxidative stress. PdCrz1 also controls cell wall integrity and the expression of calcineurin-dependent genes such as PMR1 and PMC1 (Zhang et al., 2013). Although the target genes of Crz1 in phytopathogenic fungi are not yet known, the transcriptomic analysis of *C. neoformans* under thermal stress showed that 4%–6.8% of the differentially expressed Crz1-responsive genes were involved in redox homeostasis; these included FAD-dependent oxidoreductase, ferric reductases, ferroxidases, and laccases (Chow et al., 2017).

A BATTLE WITHIN: HOW DO FUNGAL PATHOGENS MAINTAIN INTRACELLULAR REDOX BALANCE?

Successful fungal phytopathogens overcome the oxidative burst through transcriptional, post-translational, and metabolic reprogramming to produce antioxidant enzymes, effectors, and primary and secondary metabolites (Fountain et al., 2015, 2016a, 2016b; Zaccaria et al., 2015). Intracellular ROS detoxification and scavenging rely largely on antioxidant enzymes and metabolites such as ascorbate, glutathione (GSH), flavonoids, tocopherol, and certain alkaloids, which act as cellular redox buffers (Wang et al., 2019) (Figure 4). In addition, mitochondrial pyruvate dehydrogenase kinase (PDK) reduces acetyl coenzyme-A production, which is important for cellular respiration, by inhibiting pyruvate dehydrogenase (PDH) activity. Yeast and *F. graminearum* mutants defective in PDK1 accumulate more ROS and are highly sensitive to H_2O_2-induced oxidative stress by virtue of lower mitochondrial respiration (Gao et al., 2016). In fungi, the ROS-scavenging machinery has been well studied and was recently discussed in detail (Segal and Wilson, 2018). These antioxidant systems are briefly discussed below.

Enzyme-mediated ROS neutralization

Superoxide dismutase, catalase, and peroxidases

Superoxide dismutases (SODs), a group of metallo-proteins, convert superoxide and hydroxyl radicals to oxygen and H_2O_2. The exogenous H_2O_2 is further neutralized to dioxygen and water by catalases (CATs) and peroxidases (Figure 4). In eukaryotes, cytosolic Cu,Zn-SOD, mitochondrial Mn-SOD, and an extracellular Cu,Zn-SOD have been reported (Fridovich, 1997). The deletion of SOD1 in the mutualistic fungus *Oidiodendron maius* renders it sensitive to host-generated ROS and incapable of colonizing host roots (Abba et al., 2009). CATs preferentially scavenge external H_2O_2 to protect the fungus from host-generated ROS (DeLuca et al., 1995; Zhu et al., 2020). The overproduction of CATs in *Penicillium chrysogenum* makes it resistant to higher H_2O_2 concentrations (Emri et al., 1999). Moreover, *C. purpurea* knockouts of CpTF1 (a regulator of catalase activity) exhibit reduced virulence, as all catalase activity is suppressed (Nathues et al., 2004). In yeast, five independent genes produce peroxiredoxins (three cytosolic AHP1, TSA2, TSA1, one nuclear-localized Dot5, and one mitochondrial Prx1) (Figure 4). TSA1 is known to have a vital role, as its mutant cannot be complemented by any other gene (Park et al., 2000; MacDiarmid et al., 2013). Glutathione and ascorbate peroxidases protect *B. cinerea* from intra- and extracellular peroxides and contribute to increased tolerance of higher H_2O_2 levels (Gil-ad et al., 2000; Mayer et al., 2001). *M. oryzae* deletion mutants of the fungal-specific protein DES1 (*Δmodes1*), MoAP1 (*Δmoap1*), MoAP1-regulated oxidoreductase MoTRX2 (*Δmotrx2*), and protein phosphatase MoYVH1 (*Δmoyvh1*) show enhanced ROS accumulation, downregulation of defense-related genes, and reduced fungal virulence during host infection.

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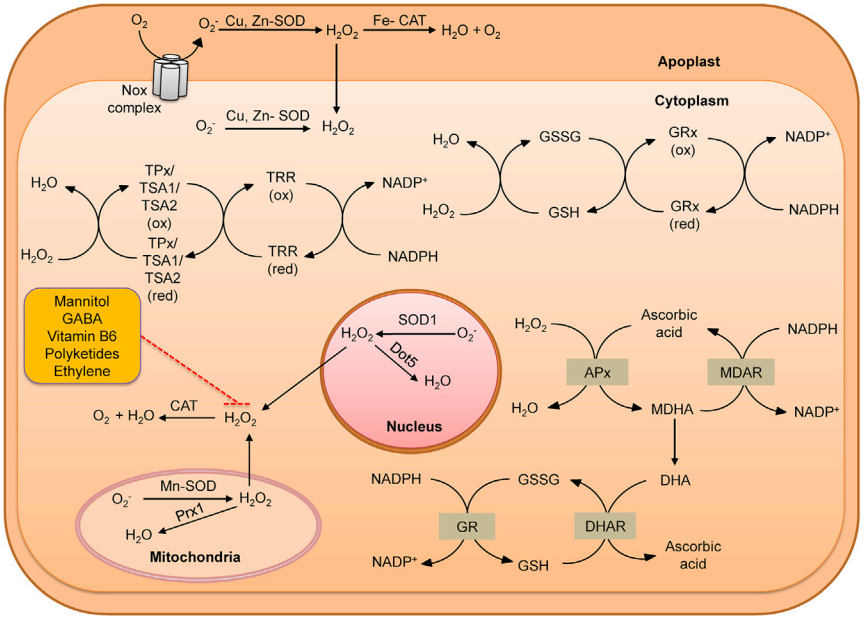

Figure 4. Fungal machinery to maintain intracellular redox homeostasis.

Under oxidative stress, physiological redox balance is maintained by various antioxidant systems such as SOD, CAT, and PRX, localized in various cellular compartments. For example, Dot5 and Prx1 are nuclear and mitochondrial peroxidases, respectively. Fungal Nox complexes produce superoxide radicals in the extracellular space; these are immediately converted to H_2O_2 by Cu,Zn-SOD, and Fe-CAT finally decomposes H_2O_2 into water and oxygen. Glutathione and thioredoxin systems are major contributors to cytosolic H_2O_2 neutralization. Glutathione reacts with H_2O_2 and forms oxidized glutathione (GSSG), which is then reduced by glutaredoxins in an NADPH-dependent manner. Similarly, thioredoxin peroxidases (Tpx) such as TSA1 and TSA2 neutralize H_2O_2 and become oxidized; the reduced Tpx form is restored by thioredoxin reductases (TRR), which draw reducing equivalents from NADPH. Ascorbate peroxidases (Apx) couple ascorbic acid with H_2O_2 and generate monodehydroascorbate (MDHA), which is interconvertible with dehydroascorbate (DHA). MDHA is converted to ascorbic acid by the NADPH-dependent monodehydroascorbate reductase (MDAR) enzyme, whereas DHA conversion to ascorbic acid by DHA reductase (DHAR) is glutathione dependent. Apart from enzymatic antioxidants, a few non-enzymatic antioxidants such as mannitol, γ-aminobutanoic acid (GABA), vitamin B6, polyketides, and ethylene are also known to reduce intracellular ROS; however, their mechanisms of action remain unclear.

The reduced production or activation of peroxidases and laccases is believed to be a reason for the active accumulation of ROS (Chi et al., 2009; Wang et al., 2017; Kou et al., 2019). In addition, peroxidase-generated ROS increase cuticular permeability, which aids in necrotrophic fungal invasion (Survila et al., 2016).

Thioredoxins and glutaredoxins

Thioredoxins (Trx; 12 kDa) are well-conserved components of the basal ROS-scavenging machinery that contains a dithiol-disulfide active site (Fernandez et al., 2014). Trx and NADPH-dependent thioredoxin reductase (TrxR) together form the thioredoxin system, which functions ubiquitously to sustain redox homeostasis. The active cysteine residues are oxidized to form disulfide (Trx-S_2) and are further reduced to dithiol (Trx-$(SH)_2$) in a TrxR-dependent manner (Zhang et al., 2016b) (Figure 4). In *M. oryzae*, thioredoxin peroxidases (Tpx1), thioredoxin reductase (Trr1), and Trx2 are essential for host invasion and intracellular ROS metabolism (Fernandez et al., 2014). The *Δmotrx2* mutants are defective in ROS scavenging and suppression of rice basal immunity. These studies show that the functions of MoTrx2 and MoAP1 overlap in stress response and pathogenicity, indicating that MoAP1 mediates the transcriptional regulation of MoTrx2 (Wang et al., 2017). Similarly, deletion of FgTRR hampers deoxynivalenol (DON) production, increases sensitivity to oxidative stress, promotes the accumulation of intracellular ROS, and thereby causes apoptosis-like death of *F. graminearum* (Fan et al., 2019).

The glutathione antioxidant system involves small ubiquitous proteins called glutaredoxins, which are necessary for ROS scavenging. Glutaredoxins are similar to Trx, except that the neutralization of ROS is performed non-enzymatically by glutathione (GSH), a small Glu-Cys-Gly tripeptide. In the presence of ROS, NADPH contributes electrons to oxidize GSH and form glutathione disulfide (GSSG), which is then recycled to reduced GSH in a glutathione reductase (Gtr)-dependent manner (Segal and Wilson, 2018; Wang et al., 2019) (Figure 4). The *Δgtr1* mutant of *M. oryzae* accumulates high levels of H_2O_2 at the infection site, suggesting that Gtr1 has a crucial role in quenching host-generated ROS during the early stages of rice–*Magnaporthe* interactions (Fernandez et al., 2014; Wang and Wang, 2018).

Non-enzymatic antioxidants

There is very scant information available about antioxidant metabolites and peptides in fungal phytopathogens. In *Rhizoctonia solani* pathotype AG3, the vitamin B6 biosynthetic pathway genes RsolPDX1, RsolPDX2, and RsolPLR are differentially expressed in response to ROS-generating compounds, paraquat, and H_2O_2. The products of the vitamin B6 biosynthesis pathway are well-known antioxidant metabolites and ROS quenchers (Samsatly et al., 2018). *F. graminearum* double knockouts (*Δgta1Δgta2*) of pyridoxal-dependent γ-aminobutanoic acid (GABA) transaminases (GTAs) show increased sensitivity to host-generated ROS, as well as reduced virulence. These findings point toward a crucial role of the GABA shunt in intracellular redox homeostasis (Bönnighausen et al., 2015). Ascorbic acid, a well-known antioxidant, is essential for sclerotial differentiation under high oxidative stress conditions in *S. rolfsii* (Georgiou et al., 2003). Moreover, various phytopathogenic fungi produce mannitol, which may serve as both an osmolyte and a powerful ROS quencher. For example, *A. alternata* produces enormous amounts of mannitol to neutralize the effect of the oxidative burst during its interaction with host plants (Vélëz et al., 2008; Meena et al., 2015) (Figure 4).

The four transcription factors AtfB, SrrA, Ap-1, and MsnA drive the induction of aflatoxin synthesis in response to oxidative stress in *A. parasiticus* (Hong et al., 2012). There is no direct evidence

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that aflatoxin scavenges ROS; however, its biosynthetic pathway utilizes oxygen-rich molecules and hence reduces oxidation (Jayashree and Subramanyam, 2000; Fountain et al., 2018). Deletion of the catalase1 gene (*cta1*) causes excess ROS accumulation, which impedes the redox homeostasis necessary for aflatoxin synthesis (Zhu et al., 2020). Recent evidence suggests that aflatoxin synthesis also generates ROS such as superoxides and H_2O_2 in *A. parasiticus* (Roze et al., 2015; Zhao et al., 2018). In *Aspergillus flavus*, ethylene inhibits aflatoxin synthesis by reducing ROS production, lipid peroxidation, and glutathione homeostasis (Huang et al., 2009) (Figure 4).

A WAR OUTSIDE THE FUNGUS AND INSIDE THE HOST: EFFECTOR-CENTRIC EXTRACELLULAR TACTICS TO BALANCE REACTIVE OXYGEN SPECIES

In addition to intracellular ROS scavenging, the modulation of host ROS production is another fascinating mechanism adapted by many specialized fungal pathogens to combat the oxidative burst. In a sophisticated defense strategy, fungi deploy a unique arsenal of small secreted proteins (SSPs) called "effectors" (Prasad et al., 2019; Van de Wouw and Idnurm, 2019). Fungal effectors are mostly small serine- or glycine-rich proteins, whereas apoplastic effectors are generally cysteine rich (Sperschneider et al., 2015; Nishimura et al., 2016). Fungal effectors exhibit less homology with other proteins, and interestingly some are non-proteinaceous (Fouche et al., 2018; Collemare et al., 2019; Van de Wouw and Idnurm, 2019; Feldman et al., 2020). The expression of effector genes is tightly regulated, as they have specific functions to perform throughout the infection phase (Sanchez-Vallet et al., 2018). For example, *M. oryzae* secretes the catalase-peroxidase CPXB to prevent ROS accumulation in rice epidermal cells during early infection (Tanabe et al., 2011). Once secreted into the host cell, effectors are capable of remodeling the host's transcriptomic and metabolic responses (Lo Presti et al., 2015). Moreover, not all pathogen-encoded effectors contribute to virulence, with the exception of a few vital core effectors such as Avr-effectors, which markedly knock down the plant basal immune system (Dangl et al., 2013; Fujisaki et al., 2015). The various roles of effectors with respect to the fungal lifestyle or phase-specific ROS homeostasis are discussed here (Figure 5).

Hide and seek: the escape strategy of endophytic and biotrophic fungi

Our current understanding of biotrophic plant–pathogen interactions sheds light on the evolution of fungal strategies to suppress the host-generated oxidative burst by targeting various subcellular and suborganellar compartments engaged in active ROS generation (Figure 5A). In plants, powdery mildews, smuts, and rusts are the common diseases caused by biotrophic fungi, which obtain their nourishment from a living host (Lorrain et al., 2018; Lorrain et al., 2019). They use unique strategies to decisively dampen basal immunity while keeping the host barely alive in order to complete their parasitic lifecycle. For example, the causal agent of cucurbit powdery mildew, *Podosphaera xanthii*, is known to produce over 50 effectors called *Podosphaera* effector candidates (PECs). The *pec019* and *pec032* mutants produced by host-induced gene silencing show high accumulation of host-generated ROS (Martinez-Cruz et al., 2018). Similarly, *Puccinia* effector candidate 6 (PEC6) is highly expressed during infection and is secreted from the haustoria into the host cytosol, where it suppresses PTI in *Nicotiana*, *Arabidopsis*, and *Triticum* (Figure 5A). Interestingly, PEC6 can also suppress ROS accumulation and chlorosis elicited by *Pseudomonas* spp. (Liu et al., 2016). PEC6 is a small cysteine-rich effector; hence, we cannot entirely rule out the possibility of its apoplastic function (Sperschneider et al., 2015).

The *U. maydis* protease inhibitor effector Pep1 surrounds fungal hyphae in the apoplast and interacts with maize peroxidase POX12, which functions in plant defense. The *Δpep1* mutants of *U. maydis* and *Ustilago hordei* face robust host defenses such as H_2O_2 accumulation and PCD; they are defective in penetrating through host epidermal cells and spreading further into neighboring cells. This evidence suggests that Pep1 is helping the invading hyphae by scavenging host ROS in the apoplast (Hemetsberger et al., 2012; Giraldo and Valent, 2013; Hemetsberger et al., 2015; Lanver et al., 2017; Wang and Wang, 2018; Zuo et al., 2019; Rocafort et al., 2020) (Figure 5A). In addition, the *Δgas1* and *Δgls1* double mutants of *U. maydis* that lack glucosidase II and glucosidase I, respectively, elicit an enormous amount of plant ROS production, indicating that *N*-glycosylation of SSPs has a critical role in the establishment of biotrophy (Fernandez-Alvarez et al., 2013; Lo Presti et al., 2015).

Evidence suggests that effectors can change the structural and functional conformation as well as the localization of host target proteins that are key factors in ROS production. For example, the Pst_12806 effector produced by *P. striiformis* f. sp. *tritici* (*Pst*) is translocated to the chloroplast and interferes with photosynthetic reactions to prevent localized host cell death (Figure 5A). In the chloroplast, Pst_12806 interacts with TaISP proteins, which are components of the cytochrome b_6/f complex. The Pst_12806–TaISP interaction takes place at the Rieske domain characterized by a [2Fe–2S] cluster at the C-terminus of the TaISP protein. The [2Fe–2S] cluster is coordinated by two cysteine and histidine residues, which transfer electrons to a heme group of cytochrome *c*. It is thought that the binding of Pst_12806 to the Rieske domain may perturb the electron transfer process during photosynthesis, thereby curtailing photosynthesis and the accompanying ROS accumulation (Xu et al., 2019) (Figure 5A). The recently reported chloroplast targeting protein 1 (CTP1) produced by *Melampsora larici-populina*, is another well-known example of such a compartment-specific effector (Lorrain et al., 2018). A glycine- and serine-rich effector, PstGSRE1 is highly induced during the early stages of *P. striiformis* infection in wheat plants and is also known to suppress the PCD triggered by Bax together with elicitor-like protein Pst322, which functions in fungal virulence. This effector targets the ROS-associated LSD-1-like zinc finger transcription factor TaLOL2, which is a critical component of the ROS signaling pathway and acts as a positive regulator of wheat immunity. The interaction disrupts the nuclear localization of TaLOL2, thus compromising plant defense by suppressing H_2O_2 accumulation and ROS-mediated cell death (Qi et al., 2019) (Figure 5A).

On the other hand, the endophytic basidiomycetous fungus *Piriformospora indica* produces the non-specific cytosolic

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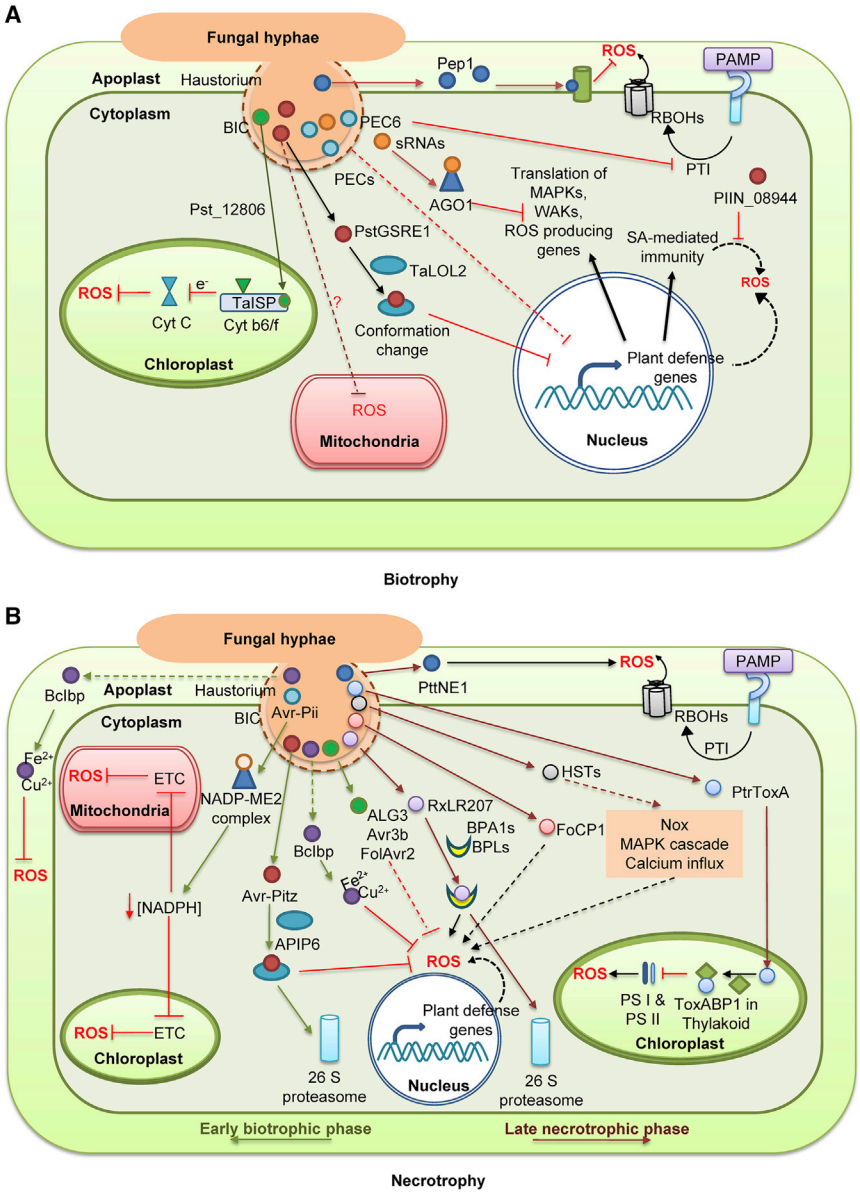

Figure 5. Effector-mediated host ROS modulation by various fungal phytopathogens.
Various effectors (small circles in the fungal cell schematic) from biotrophic (*Piriformospora indica*, *Podosphaera xanthii*, *Puccinia striiformis*, *Ustilago maydis*) and necrotrophic (*Botrytis cinerea*, *Cochliobolus victoriae*, *Fusarium oxysporum*, *Magnaporthe oryzae*, *Phytophthora capsici*, *Phytophthora sojae*, *Pyrenophora teres* f. *teres*, *Pyrenophora tritici-repentis*) phytopathogens depicted in this figure manipulate host immunity for successful infection. Because the orthologs of these effectors are not conserved among pathogenic fungi, it would be exciting to elucidate similar mechanisms in other fungi.
(A) Biotrophy-associated effectors suppress PTI as well as ROS production to keep the host alive as a continuing source of nourishment. Cytoplasmic effectors may target proteins in the cytosol or in subcellular organelles such as chloroplasts and mitochondria. For example, the wheat pathogen effector PstGSRE1 targets the cytoplasmic transcription factor TaLOL2, which is responsible for defense gene activation, whereas Pst_12806 targets the TaISP subunit of cytochrome b_6/f, which is an integral part of electron transport.
(B) During the early infection phase, necrotrophs secrete effectors to control NADPH and chelate divalent cations to reduce ROS production; at a later stage, they facilitate necrosis and promote further host ROS production to kill the host and acquire nourishment. In the chloroplast, the effector Ptr ToxA binds to ToxABP1 and impairs photosystem function, whereas cytosolic effectors such as RxLR207 target BPA1s and BPL-like proteins for 26S proteasomal degradation, leading to enhanced ROS accumulation. HSTs interfere with Nox functioning, MAPK cascades, and calcium influx to promote ROS production. Overall, during the biotrophic phase, fungal pathogens escape ROS production, whereas during the necrotrophic phase, they exploit host-produced ROS to promote PCD.

effector PIIN_08944 to colonize a wide array of plants, including *Arabidopsis* and barley. The *P. indica* effector candidate PIIN_08944 lacks a DELD motif and targets plant metabolism to promote successful colonization. It suppresses SA-mediated basal plant immunity and aids root colonization by the oomycetous biotrophic pathogen *Hyaloperonospora arabidopsidis*. The deletion of PIIN_08944 impairs the pathogen's ability to colonize *Arabidopsis* roots, whereas HvPIIN_08944 overexpression can suppress ROS accumulation and other plant defense responses in barley (Akum et al., 2015) (Figure 5A). In addition, recently discovered bidirectional cross-kingdom movement of small RNAs during plant–microbe interactions suggests that novel RNA effectors may suppress plant defense responses (Weiberg et al., 2013; Weiberg et al., 2014). For example, *B. cinerea* delivers numerous small RNAs into the host, and they are sequestered by the *Arabidopsis* Argonaute protein (AGO1). This process takes advantage of the host RNAi engine to silence defense signaling components such as MAPKs, cell-wall-associated kinases, and genes that encourage ROS accumulation (Weiberg et al., 2013; Collemare et al., 2019; Huang et al., 2019) (Figure 5A). Moreover, *M. oryzae* mutants of the tRNA-isopentenyl transferase cytokinin synthesis 1 (Cks1), which is responsible for cytokinin synthesis, encounter an enhanced oxidative burst. This indicates that cytokinin functions as a virulence effector that attenuates host defense and favors the establishment of blast disease (Chanclud et al., 2016; Shen et al., 2018).

A large number of genes encode enzymes responsible for the biosynthesis of secondary metabolites that mediate fungal virulence in Dothideomycetes. Non-ribosomal peptide synthetases (NPSs), polyketide synthases (PKSs), and terpene synthases (TPSs) are among the examples. A genome-wide study that identified the counterparts of *C. heterostrophus* NPSs, PKSs, and the less explored TPSs suggests that NPS2 is conserved in 17 out of the 18 genomes. It may have a critical function of preventing the

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Fenton reaction and, thus, the accumulation of ROS (Ohm et al., 2012). Recently, the VdNPS protein has been found to regulate the virulence of *V. dahliae* by modulating host ROS production and SA-mediated signaling (Luo et al., 2020). In addition, many fungi need to limit their exposure to toxic compounds produced during plant–fungus and fungus–microbe interactions. For example, the ATP-binding cassette (ABC) transporter ABCG29 enables the mycopathogenic fungus *Clonostachys rosea* to tolerate the influx of toxic compounds such as H_2O_2. Mutations in *abcg29* reduce H_2O_2 tolerance just like the entomopathogenic fungus *Beauveria bassiana*. Deletion of the ABC-G PDR and ABC-B multidrug resistance transporters in *B. cinerea* and *M. oryzae*, respectively, also has the same effect (Dubey et al., 2016). These "escape" strategies are deployed mainly by biotrophic or endophytic pathogens, as they need the host to be alive yet defenseless.

Rise and conquer: the exploitation strategy of necrotrophic fungi

Unlike biotrophic fungi, necrotrophs do not require a living host to prosper, and they employ strategies to kill the host. It is currently thought that all necrotrophic pathogens have an initial biotrophy-like infection phase during which they escape host-generated ROS. For example, *Zymoseptoria tritici* infection on wheat plants occurs in four distinct stages. The first stage is characterized by hyphal penetration of the leaf tissue. The second stage includes asymptomatic biotrophic invasion of intercellular spaces between mesophyll cells, whereas the third stage is a symptomatic phase during which the transition from biotrophic to necrotrophic growth occurs and conidiogenous cells begin to grow in pycnidia. The accumulation of host-generated ROS takes place in the middle to late third stage, after which the final necrotrophic phase for the colonization of nutrient-rich, ROS accumulated hostile environment begins (Haueisen et al., 2018). This is possibly a typical case in which a necrotrophic pathogen takes advantage of the oxidative burst for its own growth and development.

Early biotrophic phase

During the biotrophic phase of *M. oryzae* infection, a large number of upregulated genes encode biotrophy-associated secreted (BAS) proteins such as BAS1, which is secreted in large amounts from the invasive hyphae into the rice cytoplasm; however, it is unclear whether they are limited to the biotrophic phase alone. However, BAS1 effectors can induce basal immunity processes such as callose deposition and ROS production in rice leaves and can promote fungal virulence (Yang et al., 2017). The secreted LysM protein and non-Avr effector Slp1 competes with chitin elicitor binding protein for binding to chitin oligosaccharides, thereby suppressing chitin-induced defense-related gene expression and ROS generation in rice (Mentlak et al., 2012). The rice blast fungus encodes another class of glycine-rich small effectors known as PWLs, including PWL1 to PWL4, which function against basal immunity in a host-specific manner (Sweigard et al., 1995; Zhang and Xu, 2014). The asparagine-linked glycosylation 3 (ALG3) effector is essential for virulence, and *Δalg3* mutants accumulate more host-generated ROS (Chen et al., 2014; Lo Presti et al., 2015) (Figure 5B). Moreover, the *M. oryzae* effector AVR-Pii inhibits *Oryza sativa* NADP-malic enzyme 2 (Os-NADP-ME2) and attenuates ROS production. A similar decrease in ROS levels was observed in *ΔOs-nadp-me2* knockout rice plants. The AVR-Pii–Os-NADP-ME2 interaction occurs at the biotrophic interface complex (BIC), reprograms the metabolism, and suppresses NADPH production, thereby impeding the electron transfer necessary for the host-generated oxidative burst (Singh et al., 2016a) (Figure 5B). In addition, the ectopic expression of the AvrPiz-t effector suppresses PAMP-triggered ROS production in transgenic rice, similar to AvrPiz-t interacting protein 6 (APIP6)-silenced plants. AvrPiz-t suppresses the ubiquitin ligase activity of the rice RING E3 ubiquitin ligase APIP6, which in turn ubiquitinates AvrPiz-t, causing the degradation of both proteins via the host 26S proteasome pathway (Park et al., 2012) (Figure 5B). Furthermore, lysine-free *AvrPiz-t* (*LF-AvrPiz-t*) accumulates in the cytoplasm when expressed transiently, mainly because of its inability to bind to the APIP proteins that are positive regulators of PTI. The small GTPase homolog OsRac1, which is involved in the defense-related oxidative burst, interacts with both AvrPiz-t and LF-AvrPiz-t, and it may therefore function as an effector target to manipulate host ROS production (Bai et al., 2019).

Interestingly, the transient expression of the *P. sojae* effector Avr3b in *Nicotiana benthamiana* reduces ROS accumulation near the invasion sites and supports early phases of host colonization by *Phytophthora parasitica* and *Phytophthora capsici* (Dong et al., 2011). In *B. cinerea*, a family of iron-binding SSPs called BcIbp can detoxify ROS produced by *Arabidopsis* (Figure 5B). This may be due to the binding of exogenous metals to BcIbp, which limits intracellular metal accumulation and prevents ROS formation (Liu et al., 2019a). Another specialist necrotroph, *F. oxysporum* sp. *lycopersici* (*Fol*), produces the Avr2 effector to target evolutionarily conserved basal immunity and suppress flg22-induced ROS production. The interacting partner of Avr2 is unknown (Di et al., 2017). In addition to secreted effectors, a recent study showed that a nitronate monooxygenase (NMO2) enzyme catalyzes the oxidative denitrification of nitroalkanes to protect *M. oryzae* from RNS. During infection, the *Δnmo2* mutants encountered a strong host oxidative burst that triggered innate immune responses in rice. More importantly, the inability to suppress the oxidative burst interfered with the formation of the effector-secreting BIC, which was restored in *Δnmo2* mutants by quenching ROS to maintain redox balance (Marroquin-Guzman et al., 2017).

Late necrotrophic phase

After the transition from the biotrophic to the necrotrophic phase, the pathogen's strategy seems to take advantage of the host oxidative burst and facilitate necrosis and PCD. Also during this phase, effectors are a trump card used to target host proteins and impede ROS homeostasis. For example, the transient expression of *M. oryzae* effectors (MoCDIP1 to MoCDIP5) induces cell death in rice protoplasts and *N. benthamiana*, suggesting that they have a role during the necrotrophic phase (Chen et al., 2013). Although fungi and oomycetes belong to different kingdoms, they share similar lifestyles and infection strategies. For instance, effector entry into the host plant is mediated by an RxLR (Arg-any amino acid-Leu-Arg) motif in oomycetes and a more degenerated, RxLR-like ([RHK]X[LMIFYW]) motif in fungi (Kale, 2012; Liu et al., 2019b). The oomycetes blight pathogen *P. capsici* produces numerous effectors such as RxLR207 to overcome plant defense mechanisms (Figure 5B). In *Arabidopsis thaliana*, ROS-mediated defense is controlled by BPA1 (binding partner of ACD11, whose deletion induces accelerated cell death) and its close homologs such as BPLs

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and BPA1-like proteins. The *Δbpa1* and *Δbpls* deletion mutants exhibit enhanced ROS production under both biotic and abiotic stresses. RxLR207 binds to BPA1 and BPLs, degrading them to promote ROS accumulation and the activation of PCD to further exploit host resources (Li et al., 2019a) (Figure 5B). Another necrotrophy-promoting effector, FoCP1, is a cysteine-rich effector of the cerato-platanin protein family produced by *F. oxysporum* f. sp. *cubense*. The transient infiltration of FoCP1 into tobacco leaves triggers strong ROS accumulation, which is required to induce PCD in host plants (Li et al., 2019b) (Figure 5B). The apoplastic effector PttNE1 from *Pyrenophora teres* f. *teres* (the causal agent of barley net-form net blotch disease) causes high levels of H_2O_2 accumulation and increases electrolyte leakage in the apoplast of susceptible HECTOR plants, as compared with the resistant NDB112 variety, to promote necrosis (Liu et al., 2015b) (Figure 5B).

In addition, pathogenic necrotrophs secrete another set of proteinaceous secondary metabolites called host-selective mycotoxins (HSTs). HSTs promote ROS accumulation to enhance PCD by either the activation of host Nox or MAPK signaling involving Ca^{2+} influx (Figure 5B). AK-toxin and AAL-toxins produced by *A. alternata*, Ptr ToxA and Ptr ToxB produced by *Pyrenophora tritici-repentis*, SnToxA produced by *Stagonospora nodorum*, and victorin produced by *Cochliobolus victoriae* are some examples of HSTs (Petrov et al., 2018). *F. graminearum* produces the mycotoxin, DON, which is a virulence factor induced by ROS (Nguyen et al., 2013; Fan et al., 2019). In wheat, Ptr ToxA interacts with ToxA binding protein (ToxABP1), a homolog of *Arabidopsis* thylakoid formation 1 (Thf1), and elicits an ROS response that reduces photosystem I and photosystem II levels (Kretschmer et al., 2019) (Figure 5B). Together, these adaptive strategies assist in necrotrophic fungal pathogenesis and promote host cell death through exploitation of the plant immune system.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Currently, the role of ROS-mediated signaling in the regulation of an array of cellular processes is taking center stage. Although the complete pathway has not yet been elucidated, ROS as signaling molecules appear to be vital for polarized hyphal growth, differentiation, development, and fungal virulence (Figure 1). However, large amounts of endogenous or host-produced ROS cause redox imbalance and lead to deleterious effects (Figure 2). In the last decade, the adaptive responses of filamentous fungi under oxidative stress conditions have been extensively investigated, and complex survival mechanisms have emerged (Figures 3 and 4). The mechanisms of survival and infection are not functionally conserved among fungal phytopathogens, whereas the basal ROS defense machinery is similar. In addition to the robust intracellular ROS neutralizing machinery, fungal phytopathogens also possess the ability to manipulate host ROS production via secreted effectors during host colonization (Figure 5). Together, these findings indicate that fungal phytopathogens employ unique survival hacks to combat the host-generated oxidative burst. Our current understanding does not allow us to make generalizations about the oxidative stress responses of pathogenic fungi owing to their vast range of hosts and lifestyles. One of the major factors responsible for this may be the unidirectional studies that focus on either the plant's defense responses or the pathogen's defense. Hence, the role of fungal oxidative stress responses during natural infection may be misinterpreted. Obtaining a comprehensive picture of compatible host–pathogen interactions will require an integrated approach in which both sides of the coin are considered in order to draw biologically relevant conclusions.

The different combat strategies used by various phytopathogens are the outcome of thousands of years of co-evolution with their host plants, which sometimes also result in host jump. Therefore, more investigations are required to reveal evolutionary aspects of the advanced strategies deployed by phytopathogenic fungi, plant-associated endophytes, and other beneficial fungi. Moreover, horizontal comparisons will also be helpful for gaining a deeper understanding of the molecular biology of plant–pathogen interactions with regard to redox management. Currently, plant–fungus interaction research has shifted focus to secreted effectors, and a great deal of molecular information is being gathered about their functions. A number of effectors discussed here (Figure 5) modulate the PTI response, but only a few specifically target the ROS-producing or ROS-scavenging machinery. The availability of high-throughput approaches for effector screening and *in silico* functional prediction have enabled the identification of many putative redox-targeting effectors. However, their molecular investigation remains meager. Surprisingly, the means by which non-proteinaceous effectors contribute to fungal virulence and specifically modulate host immunity remains an underexplored area of effector biology. Hence, it would be exciting to explore such effectors in order to uncover the nexus of host metabolic pathways manipulated by targeting redox homeostasis. We anticipate that recent methodological innovations in molecular biology, bioinformatics, and instrumentation will enable us to unravel the potential oxidative stress response regulators of fungal phytopathogens. Moreover, understanding the operation of these regulators in beneficial plant–microbe interactions could provide insights into how plants distinguish between symbiotic and pathogenic microbes. Systematic and integrated research in these areas would have great significance, given the future needs of our ever-growing population during times of climate change.

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