Review
The Role of the Glutathione System in Stress Adaptation, Morphogenesis and Virulence of Pathogenic Fungi

Tanaporn Wangsanut and Monsicha Pongpom *

Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
* Correspondence: monsicha.p@cmu.ac.th

Abstract: Morphogenesis and stress adaptation are key attributes that allow fungal pathogens to thrive and infect human hosts. During infection, many fungal pathogens undergo morphological changes, and this ability is highly linked to virulence. Furthermore, pathogenic fungi have developed multiple antioxidant defenses to cope with the host-derived oxidative stress produced by phagocytes. Glutathione is a major antioxidant that can prevent cellular damage caused by various oxidative stressors. While the role of glutathione in stress detoxification is known, studies of the glutathione system in fungal morphological switching and virulence are lacking. This review explores the role of glutathione metabolism in fungal adaptation to stress, morphogenesis, and virulence. Our comprehensive analysis of the fungal glutathione metabolism reveals that the role of glutathione extends beyond stressful conditions. Collectively, glutathione and glutathione-related proteins are necessary for vitality, cellular development and pathogenesis.

Keywords: glutathione; metabolism; morphogenesis; stress response; virulence; fungal pathogen

1. Introduction

The integration of metabolism with virulence has become a new paradigm in the host–fungal pathogen interaction. In addition to virulence attributes, the term “fitness attributes” has been defined as the cellular functions that are required to support microbial growth and survival [1]. The fitness attributes include the metabolic capacity to assimilate host nutrients, resistance to host-imposed stress, tolerance to elevated host temperature, and the construction of a robust cell wall [2]. The inactivation of fitness attributes will diminish the ability of a fungus to obtain nutrients or combat environmental stressors and hence, will attenuate its ability to grow, express virulence factors, and ultimately cause infection. The metabolism provides a platform for generating the precursors and energy required for growth, antioxidant production and cell wall remodeling. Moreover, metabolic adaptation can modulate the expression of virulence factors and immunogenicity. Thus, the metabolism impacts fungal pathogenicity through both virulence and fitness attributes and is indispensable for a fungal pathogen to colonize and infect a host successfully.

Fungi can infect multiple sites on the human body and cause both superficial and life-threatening infections. As one and a half million people are killed by pathogenic fungi every year, fungal pathogens are known as a “hidden killer” [3]. The most common culprits of human fungal infection come from four major fungal groups: Candida species, Cryptococcus neoformans, Aspergillus fumigatus and thermal dimorphic fungi [4]. Important human fungal pathogens belonging to the thermal dimorphic fungus group are Talaromyces marneffei, Histoplasma capsulatum, Coccidioides immitis, Paracoccidioides brasiliensis, Blastomyces dermatitidis and Sporothrix schenckii. During infection, the host’s innate immune cells, such as macrophages or neutrophils, commonly phagocytize and destroy the fungal cells by generating reactive oxygen species (ROS) and reactive nitrogen species (RNS) [5]. The host macrophages have been reported to produce up to 14 mM hydrogen peroxide and up to 57 uM nitric oxide in response to fungal infections [6,7]. To cope with these host-imposed...
stressors, fungal pathogens possess antioxidant defense systems with both enzymatic and non-enzymatic mechanisms [8]. In addition, several fungi can switch their morphology to protect themselves from the human immune system.

Morphological plasticity is one of the main virulence attributes in pathogenic fungi. Cell differentiation and development contribute to diverse morphological changes, including germination, conidiation, morphological switching between yeast and mold forms, and even autolysis. Fungal species generally undergo a morphological transformation during host colonization by responding to specific environmental cues. For example, thermal dimorphic fungi switch morphology from a multicellular mold in environmental niches to a yeast form in warm-blooded hosts due to temperature changes [9–11]. Another example is *Candida albicans*, which can be a commensal organism of the human microbiome while also being the most prevalent human fungal pathogen. At least nine distinct cell shapes have already been found in this species. *C. albicans* changes morphotypes when it inhabits different host niches or when it changes between being a commensal or pathogenic organism [12]. In *A. fumigatus*, a ubiquitous pathogenic mold, germination of conidia and hyphal growth occurs during an invasive infection of human lungs, while conidiation is strictly inhibited [13–15]. The dysregulation of these morphology pathways consistently attenuates the virulence of the pathogenic fungi in animal studies [15–18]. Overall, cell differentiation and development are critical for fungal morphogenesis and pathogenicity.

This review focuses on the integral role of glutathione metabolism in providing a platform for generating precursors for the antioxidant system and modulating morphogenesis and virulence. Our comprehensive review highlights the role of glutathione metabolism in strengthening virulence and fitness attributes in pathogenic fungi.

2. Glutathione Systems

Glutathione (L-γ-glutamylcysteinylglycine) is a crucial metabolite in eukaryotes and plays a major role in protecting cells against oxidative damage [19,20]. Glutathione directly scavenges diverse oxidants, such as superoxide anion, hydroxyl radical, nitric oxide and carbon radicals and is also a cofactor for various antioxidant enzymes, including glutathione peroxidases and glutathione S-transferase [21,22]. There are two states of glutathione in the cells: reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) (Figure 1). Importantly, GSH is a major tissue antioxidant, while GSSG is accumulated when cells are exposed to increased levels of oxidative stress. Thus, increased ratios of GSSG to GSH are indicative of oxidative stress, and cells tightly maintain levels of reduced glutathione through the balance of its synthesis and reduction.

The GSH/GSSH pathway is composed of five enzymes, which will be the major focus of this review paper. The first two enzymes are involved with glutathione de novo biosynthesis via two ATP-dependent steps: the first step is catalyzed by γ-glutamylcysteine synthetase (GSH1), while the second step is catalyzed by glutathione synthetase (GSH2) (Figure 1). Next, glutathione peroxidase and glutathione S-transferase catalyze the production of GSSH. Glutathione peroxidase is a major enzyme in the defense against hydrogen peroxide (GPx: 2GSH + ROOH → GSSG + H₂O + ROH, EC 1.11.1.9). Glutathione S-transferase is involved with the detoxification of many xenobiotic compounds by catalyzing the conjugation of substrates to GSH (GST: GSH + RX → GS-X + RH, EC 2.5.1.18), which can then be eliminated from the cells via glutathione conjugate pumps. Lastly, glutathione reductase catalyzes the regeneration of GSH (GSSG + NADPH + H⁺ → 2GSH + NADP⁺, EC 1.6.4.2).
pathogens; the role of the glutathione system is mostly inferred from gene or protein expression analyses. Therefore, we summarized the experimentally verified functions of glutathione genetic studies have not been extensively explored, as seen in model fungi and other human fungal pathogens; the role of the glutathione system is mostly inferred from gene or protein expression analyses. Therefore, we summarized the experimentally verified functions of glutathione enzymes (\( \gamma \text{-glutamylcysteine synthetase} \) and \( \text{glutathione synthetase} \)) from diverse fungal species (Table 1). This summarized table will be beneficial in predicting the gene functions for other less studied fungi, such as thermal dimorphic fungi or other non-model fungi.

3. Glutathione and Role in Oxidative Stress Protection

Molecular mechanisms elucidating how the glutathione system plays a crucial role in the fungal stress response are primarily obtained from model fungi, such as \textit{Saccharomyces cerevisiae}, \textit{Schizosaccharomyces pombe} and \textit{Aspergillus nidulans}, or common human fungal pathogens, such as \textit{C. albicans}, \textit{A. fumigatus} and \textit{C. neoformans}. In thermal dimorphic fungi, however, an understanding of the glutathione system is lacking because glutathione genetic studies have not been extensively explored, as seen in model fungi and other human fungal pathogens; the role of the glutathione system is mostly inferred from gene or protein expression analyses. Therefore, we summarized the experimentally verified functions of each homologous glutathione gene from diverse fungal species (Table 1). This summarized table will be beneficial in predicting the gene functions for other less studied fungi, such as thermal dimorphic fungi or other non-model fungi.

3.1. \( \gamma \)–Glutamylcysteine Synthetase and Glutathione Synthetase

As shown in Table 1, the deletion of the genes encoding glutathione synthetic enzymes (\( \text{GSH1} \) or \( \text{GSH2} \) genes or their homologs) resulted in fungal mutants that were glutathione auxotrophs and had an increased sensitivity to various oxidants. Interestingly, the deletion of \( \text{gshA} \) in \textit{A. fumigatus} also impaired cellular iron sensing, indicating crosstalk between glutathione biosynthesis and fungal iron homeostasis [23]. Likewise, the deletion of glutathione synthetase (\( \text{GSH2} \)) from \textit{C. neoformans} resulted in glutathione auxotrophy under iron starvation-induced stress [24]. Indeed, glutathione is proposed to be involved with iron metabolism by its requirement in the Fe–S cluster assembly [25,26]. Importantly, the \( \text{GSH1} \) and \( \text{GSH2} \) genes are essential in diverse fungal species. A study in \textit{H. capsulatum} reported that the deletion of the \( \text{GSH1} \) or \( \text{GSH2} \) genes caused non-viable mutants, indicating that both the \( \text{GSH1} \) and \( \text{GSH2} \) genes are essential. Consistent with the result from \textit{H. capsulatum}, \( \text{gshA} \) (\( \gamma \)-glutamylcysteine synthetase gene) from \textit{Aspergillus oryzae} was also reported to be an essential gene, while the role of the glutathione synthetase gene (\( \text{GSH2} \) homolog) in cellular viability has not been characterized in this fungal species [27]. In \textit{Candida glabrata}, \( \text{GSH1} \), but not \( \text{GSH2} \), was an essential gene. These results suggest that the role of the \( \text{GSH1} \) and \( \text{GSH2} \) genes differs from various species in cell viability, and glutathione synthesis has an essential role in iron homeostasis in many fungi.
Table 1. Analyses of glutathione-related enzymes in diverse fungal species.

| Enzymes in Glutathione System | Species | Gene Name | Phenotypes | References |
|-------------------------------|---------|-----------|------------|------------|
| **γ-glutamylcysteine synthetase** | Saccharomyces cerevisiae | GSH1 | The gsh1Δ mutant showed glutathione auxotrophy, slower growth and increased sensitivity to oxidative stress. | [28,29] |
|                               | Schizosaccharomyces pombe | gcs1 | - The gcs1Δ mutant showed glutathione auxotrophy and sensitivity to cadmium. - The gcs1Δ mutant was unable to sporulate. | [30,31] |
|                               | Candida albicans | GCS1 | - The gcs1Δ mutant showed glutathione auxotrophy, increased ROS production and apoptosis. - The gcs1Δ mutant showed no change in morphogenesis and virulence. | [32,33] |
|                               | Nakaseomyces glabrataa (formerly, Candida glabrata) | GSH1 | - The gsh1Δ mutant was lethal. - A conditional deletion mutant, gsh1Δpro2-4, showed low glutathione levels and slower growth in media lacking glutathione. - The gsh1Δpro2-4 mutant showed sensitivity to oxidative stress (H₂O₂, menadione) and cadmium. | [33,34] |
|                               | Histoplasma capsulatum | GSH1 | - The GSH1 gene was expressed only in the yeast form. - The gsh1Δ mutant was lethal. - The GSH1 overexpression mutant showed an inability to switch from yeast to mold form. | [35] |
|                               | Saccharomyces cerevisiae | GSH2 | The gsh2Δ and the GSH2 overexpression mutants showed normal responses to oxidative stress. | [36] |
|                               | Schizosaccharomyces pombe | gsh2 | The gsh2Δ mutant showed glutathione auxotrophy and sensitivity to cadmium. | [30,31] |
|                               | Nakaseomyces glabrataa (formerly, Candida glabrata) | GSH2 | - The gsh2Δ mutant showed glutathione auxotrophy. - The gsh2Δ mutant showed low glutathione levels and sensitivity to oxidative stress (H₂O₂, menadione) and cadmium. - The gsh2Δ mutant showed resistance to tert-butyl hydroperoxide and cumene hydroperoxide stressors. | [34] |
| **Glutathione synthetase** | Cryptococcus neoformans | GSH2 | - The gsh2Δ mutant showed glutathione auxotrophy under iron starvation conditions. - The gsh2Δ mutant showed low glutathione levels and sensitivity to the oxidative stressor diamide, but not H₂O₂. - The gsh2Δ mutant showed sensitivity to a high salt stressor, the cell wall damaging agent Congo red, and antifungal drugs. - The gsh2Δ showed impairment in virulence-related traits, including defects in capsule formation, melanin production and growth at 37 °C. | [24] |
|                               | Histoplasma capsulatum | GSH2 | - The GSH2 gene was highly expressed in the yeast form. - The GSH2 overexpression mutant showed an inability to switch from yeast to mold form. - The gsh2Δ mutant was lethal. | [35] |
Table 1. Cont.

| Enzymes in Glutathione System | Species                          | Gene Name | Phenotypes                                                                                                                                  | References |
|-------------------------------|---------------------------------|-----------|----------------------------------------------------------------------------------------------------------------------------------------------|------------|
|                               | Saccharomyces cerevisiae        | GLR1      | The glr1Δ mutant showed sensitivity to oxidative stress (H₂O₂).                                                                           | [37,38]    |
|                               | Schizosaccharomyces pombe       | pgr1      | - The pgr1 overexpression mutant showed resistance to the oxidative stressor menadione but not H₂O₂.                                      | [39]       |
|                               | Candida albicans                | GLR1      | - The glr1Δ mutant showed sensitivity to oxidative stress (H₂O₂) but not formaldehyde or nitrosative stress (NO).                         | [40]       |
|                               | Cryptococcus neoformans         | GLR1      | - The glr1Δ mutant showed normal morphology.                                                                                               | [41]       |
|                               | Aspergillus nidulans            | glrA      | - The glr1Δ mutant showed slower growth under normal conditions.                                                                           | [42]       |
|                               | Paracoccidioides brasiliensis   | GR        | The vPh18 virulent strain showed increases in both levels of the GR gene and enzymatic activity.                                             | [43]       |
Table 1. Cont.

| Enzymes in Glutathione System | Species            | Gene Name | Phenotypes                                                                 | References |
|-------------------------------|--------------------|-----------|-----------------------------------------------------------------------------|------------|
|                               |                    |           | GPX1-3 genes encoded for phospholipid hydroperoxide glutathione peroxidase. |            |
|                               |                    |           | - The GPX3 product was a major glutathione peroxidase.                       |            |
|                               |                    |           | - The GPX3 gene was constitutively expressed.                               |            |
|                               |                    |           | - The GPX1 gene expression was induced under glucose starvation.            |            |
|                               |                    |           | - The GPX2 gene expression was induced by many oxidative stressors.         |            |
|                               | Saccharomyces       | GPX1-3    | - The gpx3Δ mutant showed sensitivity to peroxides (H₂O₂ and tert-butyl hydroperoxide). | [44,45]   |
|                               | cerevisiae         |           | - The gpx1Δ and gpx2Δ mutants showed no sensitivity to oxidative stress.    |            |
|                               |                    |           | - The gpx1Δgpx2Δgpx3Δ mutant showed sensitivity to H₂O₂ and phospholipid hydroperoxide (polysaturated fatty acid linolenate 18:3). |    |
|                               |                    |           | C. albicans GPX3 (ScGPX1 homolog)                                           |            |
|                               |                    |           | - The gpx3Δ mutant (orf19.4436Δ) showed sensitivity to H₂O₂ and was defective in hyphal formation within macrophage cells. | [46,47]     |
|                               |                    |           | - The gpx3Δ mutant showed impairment in killing macrophages and Galleria mellonella. |    |
|                               |                    |           | - The gpx3Δ mutant showed normal virulence in a murine model of infection.   |            |
|                               | Cryptococcus        | GPX1, GPX2| - GPX1 and GPX2 gene expressions were induced in response to t-butylhydroperoxide and cumene hydroperoxide and repressed in response to nitric oxide. | [48]       |
|                               | neoformans          |           | - GPX2 gene expression was induced in response to the hydrogen peroxide stressor. |            |
|                               |                    |           | - The gpx1Δ and gpx2Δ mutants showed normal morphology, melanin production and capsule formation. |    |
|                               |                    |           | - The gpx1Δ and gpx2Δ mutants showed sensitivity to cumene (hydroperoxide) but not superoxide, hydrogen peroxide or nitric oxide stressors. |    |
|                               | Aspergillus         | hyr1 (ScGPX3/ HYR1 homolog)                                                |            |
|                               | fumigatus           |           | - hyr1 gene expression was upregulated in hyphae and conidia when exposed to neutrophils. | [49,50] |
|                               |                    |           | - The hyr1 gene expression was induced when exposed to H₂O₂.                |            |
|                               | Talaromyces         | gpx1 (ScGPX3/HYR1 homolog)                                                 |            |
|                               | marneffei           |           | - Gpx1 is an antigenic protein.                                              | [51,52]   |
### Table 1. Cont.

| Enzymes in Glutathione System | Species | Gene Name | Phenotypes | References |
|-------------------------------|---------|-----------|------------|------------|
| Glutathione S-transferase     | Saccharomyces cerevisiae | GTT1-2 | - GTT1 gene expression was induced during the diauxic shift and stationary phase.  
- The gtt1Δ, gtt2Δ, and gtt1Δgtt2Δ showed sensitivity to heat shock in a stationary phase and slower growth at a high temperature of 39°C.  
- The grxl△grx2△gtt1△gtt2Δ mutant showed sensitivity to xenobiotics (1-chloro-2,4-dinitrobenzene), heat and the oxidative stressors (cumene hydroperoxide and H₂O₂). | [53,54] |
| Schizosaccharomyces pombe  | gst1-3 | | - The gst1△gst2Δ and gst3Δ mutants showed sensitivity to peroxide stressors (H₂O₂ and t-butylhydroperoxide) and the antifungal drug fluconazole.  
- The gst1△gst2Δ and gst3Δ mutants showed resistance to the peroxide stressor diamide.  
- gst1, gst2, and gst3 gene expressions were induced during the stationary phase and in response to hydrogen peroxide.  
- All Gst1, 2 and 3 enzymes have glutathione transferase activity, and the Gst3 enzyme also has glutathione peroxidase activity. | [55] |
| Candida albicans             | GST2    | | - The gst2Δ mutant showed sensitivity to oxidative stress (H₂O₂).  
- GST2 gene expression was induced under nitrogen limitation.  
- The gst2Δ mutant showed defects in hyphal switching under nitrogen starvation-induced filamentous growth. | [56] |
| Aspergillus nidulans         | gstA    | | - The gstAΔ mutant showed sensitivity to the oxidant diamide, the fungicide carboxin, various xenobiotics (pyrrolnitrin and sulphanilamide), and heavy metals (selenium, silver and nickel).  
- The gstAΔ mutant showed normal growth in the presence of 1-chloro-2,4-dinitrobenzene.  
- The gstA gene expression was induced in response to xenobiotics (1-chloro-2,4-dinitrobenzene) and oxidative stress (H₂O₂). | [57] |
| Aspergillus fumigatus        | gstA, gstB, gstC | | - All gstA, B and C enzymes have both glutathione transferase and glutathione peroxidase activities.  
- The gstA and gstC genes were constitutively expressed under normal conditions, and their expression levels were inducible in response to oxidative stress (H₂O₂).  
- The expression of all gst genes was induced in response to xenobiotics (1-chloro-2,4-dinitrobenzene). | [58] |
| Paracoccidioides brasiliensis| GST1-3  | | The vPh18 virulent strain showed increased levels of the GST1-3 genes. | [43] |
| Paracoccidioides lutzii      | GST     | | GST was exclusively secreted in the yeast form. | [59] |

### 3.2. Glutathione Reductase

In addition, glutathione reductase is required for resistance to oxidative stress because the deletion of the glutathione reductase gene commonly results in fungal mutants that are sensitive to various stressors. The details of the growth and stress response defects in deletion mutants are different according to the species. For example, in S. pombe yeast, glutathione reductase is indispensable for growth, as the pgp1Δ strain was not viable due
to the accumulation of GSSG [39,60]. In *S. cerevisiae* and *C. albicans*, the *grl1Δ* mutant was viable and only showed growth defects under oxidative stress [37,38,40]. In *C. neoformans*, the *grl1Δ* mutant grew normally under normal conditions and was sensitive to only nitric oxide stress but not to peroxide stress [41]. In *A. nidulans*, the *grlAΔ* mutant exhibited growth defects, even under normal conditions, yet was still viable [42,61]. The *grlAΔ* strain of *A. nidulans* was also defective in its growth under high temperatures. Overall, these results suggest that glutathione reductase functions differently among fungal species.

### 3.3. Glutathione Peroxidase

Furthermore, glutathione peroxidase plays a crucial role in protecting fungi against oxidative stress since the absence of glutathione peroxidase gene(s) results in fungal strains that are not able to cope with various oxidants, especially peroxides. Nonetheless, distinct cellular responses to oxidative stress could be observed among fungal species. For example, the *gpx3Δ* mutants from *S. cerevisiae* and *C. albicans* were highly sensitive to H$_2$O$_2$, while the *gpx1Δ* and *gpx2Δ* mutants from *C. neoformans* were not sensitive to H$_2$O$_2$ but were sensitive to other peroxides (Table 1). Furthermore, the *gpx1Δ* and *gpx2Δ* mutants from *S. cerevisiae* and *C. albicans* did not show any defects in response to oxidative stress, and hence, *GPX3* is proposed to be the main gene encoding for glutathione peroxidase in these fungi. In *T. marneffei*, the glutathione peroxidase gene (*gpx1*; the homolog of the glutathione peroxidase *HYR1* gene from *S. cerevisiae*) was isolated as one of the antigenic proteins [51]. The expression levels of the *T. marneffei gpx1* gene were high in the pathogenic yeast form and were relatively unchanged in the conidia or mold forms. These results imply that glutathione peroxidase contributes to immunological response during *T. marneffei* infection and plays an important role in the pathogenic yeast phase. Collectively, these results suggest that glutathione peroxidase is required for the general oxidative stress defense mechanisms yet could respond distinctly to the different stressors, depending on the fungal species.

### 3.4. Glutathione S-Transferase

Glutathione S-transferase is involved in the resistance to xenobiotics because this enzyme can detoxify a broad range of harmful substances. In addition, glutathione S-transferase can also protect the cells against oxidative stress as it possesses GSH-dependent peroxidase activity. Accordingly, the glutathione S-transferase gene deletion mutants from a wide range of fungi became sensitive to both xenobiotics and various stressors. As seen in the case of other glutathione-related genes, there are differences in the glutathione S-transferase function among individual fungal species. In *S. pombe*, the *gst1Δgst2Δ* and *gst3Δ* mutants were sensitive to the antifungal drug fluconazole, suggesting the role of glutathione S-transferase in mediating drug resistance. In *C. albicans*, the GST2 gene was additionally induced under nitrogen starvation [56]. In *S. cerevisiae*, the *gtt1Δ*, *gtt2Δ*, and *gtt1Δgtt2Δ* mutants showed an increased sensitivity to heat shock or exhibited growth defects at high temperatures. In *A. nidulans*, the *gstAΔ* mutant was sensitive to heavy metals [57]. Taken together, glutathione S-transferase is an important enzyme that protects fungal cells from diverse types of substances and stressors.

### 4. The Role of Glutathione in Fungal Morphology, Cellular Development and Virulence

Morphological transformation is an important developmental process in fungi. In the case of fungal pathogens, the ability to produce conidia, germinate, and switch morphology is highly linked to virulence. Multiple signaling pathways and transcription factors tightly regulate fungal morphogenesis, and the details have been extensively discussed elsewhere [4,12,16]. However, less is known about how the glutathione pathway specifically contributes to fungal morphogenesis, cellular development, and virulence. Importantly, genetic studies (Table 1) have demonstrated that several mutants defective in glutathione pathways are not only sensitive to oxidative stress but also exhibit abnormal cellular devel-
opment. In the thermal dimorphic fungi, *H. capsulatum*, the *GSH1* and *GSH2* genes were highly expressed in the pathogenic yeast form, and the *GSH1* and *GSH2* overexpression strains were unable to switch to the mold form [35]. Consistent with this result, the *gpx1* gene from *T. marneffei* was specifically expressed in the pathogenic yeast form [51,52]. Likewise, the *gst* gene from *Paracoccidioides lutzii* was exclusively secreted in yeast but not in mycelial cells [59]. Overall, these results demonstrate that glutathione genes are highly expressed in the pathogenic yeast form of thermal dimorphic fungi, suggesting that the glutathione pathway is important during the morphological transition and infection.

The glutathione system contributes to the morphological and cellular developments, not only in thermal dimorphic fungi but also in other fungal species. In contrast to the thermal dimorphic fungi that switch to yeast during infection, *C. albicans* switches from yeast to filamentous growth in order to penetrate tissue during host infection. Accordingly, the *C. albicans gpx3Δ* mutant was defective in hyphal formation within macrophage cells, and the *gst2Δ* mutant displayed hyphal switching defects under nitrogen starvation-induced filamentous growth [46,56]. In the filamentous fungus *A. nidulans*, the *glrΔ* mutant showed conidia germination defects at high temperatures, even under non-oxidative stress conditions [61]. In *A. fumigatus*, the transcript of the *gpx3* gene is highly induced in hyphae and conidia upon exposure to human neutrophils [49]. In the pathogenic yeast, *C. neoformans*, the ability to form a capsule, produce melanin and grow at a high temperature are required for this fungal pathogen to establish infection. The *C. neoformans gsh2Δ* mutant, however, was unable to form a capsule or produce melanin [24]. The mutant also had growth defects at 37 °C [24]. In the non-pathogenic yeasts, *S. cerevisiae* and *S. pombe*, the *gsh1Δ* mutant was defective in sporulation, an important developmental process in response to nutrient starvation [31,62,63]. To conclude, the glutathione pathway is necessary for morphological and cellular development in a myriad of fungal species.

Given the central role of the glutathione system in detoxification, cellular development and the oxidative stress response, one may speculate that the glutathione system will impact the ability of fungal pathogens to invade host tissue and cause disease in humans. Based on the data collected here (Table 1), the pathogenic fungi lacking glutathione components commonly became avirulent or had attenuated virulence in macrophage-killing models and/or animal models of infections. In *C. albicans*, the *gpx3Δ* mutant showed impaired virulence in macrophage killing and *Galleria mellonella* infection models [46]. Even though the morphology of the *C. albicans gcs1Δ* mutant was unaffected, this strain was avirulent in mice [32,33]. Likewise, the *glr1Δ* mutant was less virulent, and *GLR1* overexpression showed increased virulence in *C. albicans* [40]. In *C. neoformans*, the *glr1Δ* mutant exhibited normal morphology; however, the mutant was more sensitive to macrophage killing and was avirulent in an inhalation model of mouse infection [41]. Furthermore, the *C. neoformans gpx1Δ* and *gpx2Δ* were more sensitive to macrophage killing yet were still virulent in a mouse model and displayed normal morphology [48]. In the thermal dimorphic fungi, *Paracoccidioides brasiliensis*, the gene expression and enzymatic activity of glutathione reductase demonstrated increased levels in the virulent strain [43]. Similarly, the expression of the *GST1*, *GST2* and *GST3* genes was upregulated in the virulent strain of *P. brasiliensis* [43]. These results suggest that the glutathione pathway is implicated in virulence in *Paracoccidioides* spp. To date, however, none of the glutathione genes from thermal dimorphic fungi have been experimentally validated in regards to their role in virulence during infection. In summary, the generation of glutathione mutants, together with utilizing animal models of infection, have experimentally verified the critical role of glutathione in fungal virulence.

5. Glutathione as Modulators of Fungal Virulence and Pathogenesis

As discussed comprehensively in this review, the glutathione system plays a pivotal role in fungal species. Mutants lacking components of the glutathione system demonstrate deleterious changes in the phenotypes associated with fitness and virulence. Glutathione, however, has not oftentimes been thought of as a “regulatory signal” used by pathogens
to modulate virulence traits. Recently, several reports support the role of glutathione in regulating virulence in diverse bacterial pathogens [64]. For instance, *Listeria monocytogenes*, a causative agent of listeriosis, uses both host-derived and endogenous glutathione as a critical signaling molecule to regulate the switch from saprophytic to pathogenic lifestyles. In particular, host-derived glutathione triggered increased bacterial glutathione levels. Then, the elevated bacterial glutathione allosterically bonded to the master virulence regulator PrfA, activating the expression of the virulence genes in this pathogen [65]. In *Pseudomonas aeruginosa*, an opportunistic bacterial pathogen, the bacterial-derived glutathione directly activated the global transcription factor Vfr through a reduction reaction [66]. Once Vfr is in a reduced and activated form, Vfr can regulate the expression of protein-secretion systems, which are essential for establishing infection in this bacterial pathogen. Thus, bacterial pathogens can sense glutathione levels and use glutathione to allosterically bind or reduce the disulfide bonds of a transcription factor to regulate virulence genes directly. These examples clearly demonstrate that glutathione is a critical signaling molecule that activates the virulence pathways. Whether or not fungal pathogens can use glutathione as a modulator of their transcriptional regulation to directly control virulence pathways has yet to be explored.

Several studies have provided clues that glutathione may function as a modulator of morphogenesis and virulence in fungi (Table 2). It has been previously hypothesized that intracellular glutathione levels may function as a signaling molecule because redox imbalances, intracellular ROS accumulation and antioxidant enzyme activation can initiate the cell differentiation processes, such as germination, conidiation and dimorphic transition [67]. The first evidence came from studies showing that depletion of intracellular glutathione levels during yeast to hyphal transition was commonly observed in *C. albicans* and *A. pullulans* [68–70]. These results led to the hypothesis that intracellular levels of glutathione might signal morphological switching in these fungal species. Second, in *H. capsulatum*, the *gsh1* and *gsh2* genes, which encode enzymes responsible for glutathione biosynthesis, were highly expressed in the yeast phase. The overexpression of these genes led to yeast cells being incapable of switching to the mold morphotype, implying that high amounts of glutathione could signal this fungus to remain in the yeast morphotype (Tables 1 and 2). Third, *Penicillium chrysogenum*, an industrial fungus, underwent yeast-like cell formation in autolysing cultures. During the yeast-like cell transition from the mycelium, there was a relatively high concentration of glutathione and a reductive GSH/GSSH redox balance [71]. The studies from several fungal species consistently suggest that yeast morphotype formation is correlated with high levels of glutathione. A later study in *C. albicans*, however, revealed that glutathione depletion is a consequence of, but not a regulatory signal leading to, the filamentation process [72]. Neither supplementation nor depletion of glutathione affected the ability of *C. albicans* to form hyphae. In fact, glutathione was highly consumed during *C. albicans* filamentation and led to glutathione-dependent oxidative stress. The result from *C. albicans* definitely challenges the role of glutathione as a signaling molecule in fungi, yet also emphasizes that more experiments are needed to unravel the role of glutathione as a regulatory signal in other fungi.
Table 2. Role of glutathione levels in fungal cellular development.

| Species                  | Morphological Changes  | Role of Glutathione                                                                                                                                                                                                 | Reference |
|-------------------------|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| *Candida albicans*      | Yeast-to-hyphae transition | - Intracellular glutathione levels were decreased during a yeast-to-hyphae transition due to high glutathione consumption by filamentous cells. <br> - Glutathione levels were increased in C. albicans resistant to fluconazole. | [69,70,72–74] |
| *Aureobasidium pullulans* | Yeast-to-mycelia transition | Intracellular glutathione levels were higher in yeast cells than in mycelia.                                                                                                                                             | [68]      |
| *Histoplasma capsulatum* | Yeast-to-mycelia transition | Glutathione was highly abundant in the yeast form.                                                                                                                                                                     | [35]      |
| *Coccidioides immitis*  | Yeast-to-mycelia transition | Genes related to glutathione detoxification pathways were downregulated in yeast spherule form compared to mold form.                                                                                               | [75]      |
| *Penicillium chrysogenum* | Yeast-to-mycelia transition | Intracellular glutathione levels were increased within yeast-like cells in autolysing culture.                                                                                                                       | [71]      |
| *Saccharomyces cerevisiae* | Sporulation            | - Glutathione was required for sporulation. <br> - Intracellular glutathione levels were decreased during sporulation and completely undetectable during maximum sporulation.                           | [62,63,76] |

6. Concluding Remarks

Glutathione has been called an “altruistic metabolite” in fungi because it participates in the response of cells suffering from oxidative stress [67]. Indeed, the role of glutathione is not limited to stressful conditions. A defective cell in the glutathione metabolic pathway simply cannot survive, even in stress-free situations. Several glutathione genes are considered “essential genes” because the absence of these genes leads to lethality. In pathogenic fungi, glutathione participates in cell differentiation and morphology development, linking glutathione metabolism to virulence attributes. Even though there is no doubt that glutathione is a truly altruistic compound, many questions still remain. For example, it is unclear whether glutathione can function as a regulatory signal to control the morphological switching and virulence pathways in fungi, as seen in the case of bacterial pathogens. How does glutathione metabolism affect cellular function at a global level? How does the cell co-regulate glutathione metabolism and morphogenesis? As transcriptomic and proteomic approaches, as well as CRISPR-based gene editing technology, become more available to the research community, we can study a wider range of genes, proteins and even organisms. We anticipate that with further investigations, more examples will be uncovered to demonstrate how diverse pathogens could use glutathione as a modulator of virulence or how glutathione impacts other cellular functions.

Author Contributions: Conceptualization, T.W. and M.P.; data curation, T.W.; writing—original draft preparation, T.W.; writing—review and editing, T.W. and M.P.; visualization, T.W.; supervision, M.P.; funding acquisition, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Faculty of Medicine Research Fund, grant no. 067-2561 and 153-2564.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.
Acknowledgments: We thank Ryan Gentry Williams for reading and correcting the grammar of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; the collection, analyses, or interpretation of data; the writing of the manuscript or in the decision to publish the result.

References
1. Brown, A. Integration of metabolism with virulence in *Candida albicans*. In *Fungal Genomics*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 185–203.
2. Brown, A.J.; Brown, G.D.; Netea, M.G.; Gow, N.A. Metabolism impacts upon *Candida* immunogenicity and pathogenicity at multiple levels. *Trends Microbiol.* 2014, 22, 614–622. [CrossRef] [PubMed]
3. Brown, G.D.; Denning, D.W.; Gow, N.A.; Levitz, S.M.; Netea, M.G.; White, T.C. Hidden killers: Human fungal infections. *Sci. Transl. Med.* 2012, 4, 165rv13. [CrossRef] [PubMed]
4. Kim, J.Y. Human fungal pathogens: Why should we learn? *J. Microbiol.* 2016, 54, 145–148. [CrossRef] [PubMed]
5. Gilbert, A.S.; Wheeler, R.T.; May, R.C. Fungal pathogens: Survival and replication within macrophages. *Cold Spring Harb. Perspect. Med.* 2014, 5, e019661. [CrossRef]
6. Gross, N.T.; Nessa, K.; Canner, P.; Jarstrand, C. Production of nitric oxide by rat alveolar macrophages stimulated by *Cryptococcus neoformans* or *Aspergillus fumigatus*. *Med. Mycol.* 1999, 37, 151–157. [CrossRef] [PubMed]
7. Hidalgo, H.A.; Helmke, R.J.; German, V.E.; Mangos, J.A. *Pneumocystis carinii* induces an oxidative burst in alveolar macrophages. *Infect. Immun.* 1992, 60, 1–7. [CrossRef] [PubMed]
8. Itato, P.; Santovito, G. Enzymatic and Non-Enzymatic Molecules with Antioxidant Function. *Antioxid. Redox Signal.* 2021, 10, 579. [CrossRef]
9. Edwards, J.A.; Chen, C.; Kemski, M.M.; Hu, J.; Mitchell, T.K.; Rapleye, C.A. *Histoplasma* yeast and mycelial transcriptomes reveal pathogenic-phase and lineage-specific gene expression profiles. *BMC Genom.* 2013, 14, 695. [CrossRef]
10. Nemecek, J.C.; Wüthrich, M.; Klein, B.S. Global control of dimorphism and virulence in fungi. *Science* 2006, 312, 583–588. [CrossRef]
11. Beyhan, S.; Gutierrez, M.; Voorhies, M.; Sil, A. A temperature-responsive network links cell shape and virulence traits in a primary fungal pathogen. *PLoS Biol.* 2013, 11, e1001614. [CrossRef]
12. Noble, S.M.; Gianetti, B.A.; Witchley, J.N. *Candida albicans* cell-type switching and functional plasticity in the mammalian host. *Nat. Rev. Microbiol.* 2017, 15, 98–108. [CrossRef] [PubMed]
13. Tochigi, N.; Okubo, Y.; Ando, T.; Wakayama, M.; Shinozaki, M.; Gocho, K.; Hata, Y.; Ishiwatari, T.; Nemoto, T.; Shibuya, K. Histopathological implications of *Aspergillus* infection in lung. *Mediat. Inflamm.* 2013, 2013, 809798. [CrossRef] [PubMed]
14. Mah, J.H.; Yu, J.H. Upstream and downstream regulation of asexual development in *Aspergillus fumigatus*. *Eukaryot. Cell* 2006, 5, 1585–1595. [CrossRef] [PubMed]
15. Stewart, J.I.P.; Fava, V.M.; Kerkaert, J.D.; Subramanian, A.S.; Gravelat, F.N.; Lehoux, M.; Howell, P.L.; Cramer, R.A.; Sheppard, D.C. Reducing *Aspergillus fumigatus* virulence through targeted dysregulation of the conidiation pathway. *mbio* 2020, 11, e03202-19. [CrossRef]
16. Boyce, K.J.; Andrianopoulos, A. Fungal dimorphism: The switch from hyphae to yeast is a specialized morphogenetic adaptation allowing colonization of a host. *FEMS Microbiol. Rev.* 2015, 39, 797–811. [CrossRef]
17. Lo, H.J.; Köhler, J.R.; DiDomenico, B.; Loebenberg, D.; Cacciapuoti, A.; Fink, G.R. Nonfilamentous *C. albicans* mutants are avirulent. *Cell* 1997, 90, 939–949. [CrossRef]
18. Thompson, D.S.; Carlisle, P.L.; Kadosh, D. Coevolution of morphology and virulence in *Candida* species. *Eukaryot. Cell* 2011, 10, 1173–1182. [CrossRef]
19. Carlos, I.Z.; Silva Monnazzi, L.G.; Falcão, D.P.; Machado de Medeiros, B.M. TNF-alpha, H$_2$O$_2$ and NO response of peritoneal macrophages to *Versinia enterococolítica* O3 derivatives. *Microbes Infect.* 2004, 6, 207–212. [CrossRef]
20. Penninckx, M.J. An overview on glutathione in *Saccharomyces* versus non-conventional yeasts. *FEMS Yeast Res.* 2002, 2, 295–305. [CrossRef]
21. Grant, C.M. Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions. *Mol. Microbiol.* 2001, 39, 533–541. [CrossRef]
22. Pizzorno, J. Glutathione! *Integr. Med. (Encinitas)* 2014, 13, 8–12. [PubMed]
23. Misslinger, M.; Lechner, B.E.; Bacher, K.; Haas, H. Iron-sensing is governed by mitochondrial, not by cytosolic iron-sulfur cluster biogenesis in *Aspergillus fumigatus*. *Metallomics* 2018, 10, 1687–1700. [CrossRef]
24. Attarian, R. Analysis of the Roles of a Monothiol Glutaredoxin and Glutathione Synthetase in the Virulence of the AIDS-Associated Pathogen *Cryptococcus neoformans*. Ph.D. Thesis, University of British Columbia, Vancouver, BC, Canada, 2016. [CrossRef]
25. Kumar, C.; Igbaria, A.; D’Auteaux, B.; Planson, A.G.; Junot, C.; Godat, E.; Bachhawat, A.K.; Delaunay-Moisans, A.; Toledano, M.B. Glutathione revisited: A vital function in iron metabolism and ancillary role in thiol-redox control. *EMBO J.* 2011, 30, 2044–2056. [CrossRef] [PubMed]
26. Berndt, C.; Lilig, C.H. Glutathione, glutaredoxins, and iron. *Antioxid. Redox Signal.* 2017, 27, 1235–1251. [CrossRef]
27. Hattori, R.; Tada, S.; Matsushita-Morita, M.; Suzuki, S.; Kusumoto, K.-I. Gamma-glutamylcysteine synthetase gene homolog (gshA) is important in glutathione homeostasis in *Aspergillus oryzae*. *Jpn. Agric. Res. Q.* JARQ 2018, 52, 301–305. [CrossRef]

28. Grant, C.M.; Maclver, E.H.; Dawes, I.W. Glutathione is an essential metabolite required for resistance to oxidative stress in the yeast *Saccharomyces cerevisiae*. *Curr. Genet.* 1996, 29, 511–515. [CrossRef]

29. Grant, C.M.; Perrone, G.; Dawes, I.W. Glutathione and catalase provide overlapping defenses for protection against hydrogen peroxide in the yeast *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 1998, 253, 893–898. [CrossRef]

30. Mutoh, N.; Hayashi, Y. Isolation of mutants of *Schizosaccharomyces pombe* unable to synthesize cadyspin, small cadmium-binding peptides. *Biochem. Biophys. Res. Commun.* 1988, 151, 32–39. [CrossRef]

31. Chaudhuri, B.; Ingavale, S.; Bachhawat, A.K. The Role of the Cysteine/Glutathione Regulatory Genes. [CrossRef] [PubMed]

32. Baek, Y.U.; Kim, Y.R.; Yim, H.S.; Kang, S.O. Disruption of gamma-glutamylcysteine synthetase results in absolute glutathione auxotrophy and apoptosis in *Candida albicans*. *FEBS Lett.* 2004, 556, 47–52. [CrossRef]

33. Yadav, A.K.; Desai, P.R.; Rai, M.N.; Kaur, R.; Ganesan, K.; Bachhawat, A.K. Glutathione biosynthesis in the yeast pathogens *Candida glabrata* and *Candida albicans*: Essential in *C. glabrata*, and essential for virulence in *C. albicans*. *Microbiolology (Reading)* 2011, 157, 484–495. [CrossRef] [PubMed]

34. Gutiérrez-Escobedo, G.; Orta-Zavalza, E.; Castaño, I.; De Las Peñas, A. Role of glutathione in the oxidative stress response in the fungal pathogen *Candida glabrata*. *Curr. Genet.* 2013, 59, 91–106. [CrossRef] [PubMed]

35. Adams, M.A. The Role of the Cysteine/Glutathione Regulatory Genes *GDO1*, *GSH1*, and *GSH2* in Yeast-Mold Dimorphism of the Pathogenic Fungus *Histoplasma capsulatum*. Ph.D. Thesis, University of Southern Mississippi, Hattiesburg, MS, USA, 2012.

36. Grant, C.M.; Maclver, F.H.; Dawes, I.W. Glutathione synthetase is dispensable for growth under both normal and oxidative stress conditions in the yeast *Saccharomyces cerevisiae* due to an accumulation of the dipeptide gamma-glutamylcysteine. *Mol. Biol. Cell* 1997, 8, 1699–1707. [CrossRef] [PubMed]

37. Collinson, L.P.; Dawes, I.W. Isolation, characterization, and overexpression of the yeast gene, GLR1, encoding glutathione reductase. *Gene* 1995, 156, 123–127. [CrossRef]

38. Grant, C.M.; Collinson, L.P.; Roe, J.H.; Dawes, I.W. Yeast glutathione reductase is required for protection against oxidative stress and is a target gene for yAP-1 transcriptional regulation. *Mol. Microbiol.* 1996, 21, 171–179. [CrossRef]

39. Lee, J.; Dawes, I.W.; Roe, J.H. Isolation, expression, and regulation of the *pgr1* (+) gene encoding glutathione reductase absolutely required for the growth of *Schizosaccharomyces pombe*. *J. Biol. Chem.* 1997, 272, 23042–23049. [CrossRef]

40. Tillmann, A.T.; Strijbis, K.; Cameron, G.; Radmaneshfar, E.; Thiel, M.; Munro, C.A.; MacCallum, D.M.; Distel, B.; Gow, N.A.; Brown, A.J. Contribution of Fdh3 and Glr1 to Glutathione Redox State, Stress adaptation and virulence in *Candida albicans*. *PLoS ONE* 2015, 10, e0126940. [CrossRef] [PubMed]

41. Missall, T.A.; Pusateri, M.E.; Donlin, M.J.; Chambers, K.T.; Corbett, J.A.; Lodge, J.K. Posttranslational, translational, and transcriptional responses to nitric oxide stress in *Cryptococcus neoformans*: Implications for virulence. *Eukaryot. Cell* 2006, 5, 518–529. [CrossRef]

42. Sato, I.; Shimizu, M.; Hoshino, T.; Takaya, N. The glutathione system of *Aspergillus nidulans* involves a fungus-specific glutathione S-transferase. *J. Biol. Chem.* 2009, 284, 8042–8053. [CrossRef]

43. Castilho, D.G.; Navarro, M.V.; Chaves, A.F.A.; Xander, P.; Xander, P.; Batista, W.L. Recovery of the *Saccharomyces cerevisiae* transcription factor and macrophage escape. *Antioxid. Redox Signal.* 2013, 19, 2244–2260. [CrossRef]

44. Avery, A.M.; Avery, S.V. *Saccharomyces cerevisiae* expresses three phospholipid hydroperoxide glutathione peroxidases. *J. Biol. Chem.* 2001, 276, 33730–33735. [CrossRef] [PubMed]

45. Inoue, Y.; Matsuda, T.; Sugiyama, K.; Iwata, S.; Kimura, A. Genetic analysis of glutathione peroxidase in oxidative stress response of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 1999, 274, 27002–27009. [CrossRef] [PubMed]

46. Patterson, M.J.; McKenzie, C.G.; Smith, D.A.; da Silva Dantas, A.; Sherston, S.; Veal, E.A.; Morgan, B.A.; MacCallum, D.M.; Erwig, L.P.; Quinn, J. Ybp1 and Gpx3 signaling in *Candida albicans* govern hydrogen peroxide-induced oxidation of the Cap1 transcription factor and macrophage escape. *Antioxid. Redox Signal.* 2013, 19, 2244–2260. [CrossRef]

47. Miramón, P.; Dunker, C.; Kasper, L.; Jacobsen, I.D.; Barz, D.; Kurzai, O.; Hube, B. A family of glutathione peroxidases contributes to oxidative stress resistance in *Candida albicans*. *Mol. Biol. Med.* 2014, 52, 223–239. [CrossRef] [PubMed]

48. Missall, T.A.; Cherry-Harris, J.F.; Lodge, J.K. Two glutathione peroxidases in the fungal pathogen *Cryptococcus neoformans* are expressed in the presence of specific substrates. *Microbiology (Reading)* 2005, 151, 2573–2581. [CrossRef]

49. Sugui, J.A.; Kim, H.S.; Zaremba, K.A.; Chung, Y.C.; Gallin, J.I.; Nierman, W.C.; Kwon-Chung, K.J. Genes differentially expressed in conidia and hyphae of *Aspergillus fumigatus* upon exposure to human neutrophils. *PLoS ONE* 2008, 3, e2655. [CrossRef]

50. Fan, Z.; Yu, H.; Guo, Q.; He, D.; Xue, B.; Xie, X.; Yokoyama, K.; Wang, L. Identification and characterization of an anti-oxidative stress-associated mutant of *Aspergillus fumigatus* transformed by *Agrobacterium tumefaciens*. *Mol. Med. Rep.* 2016, 13, 2367–2376. [CrossRef]

51. Pongpom, M.; Vanittanakom, N. Stress adaptation in *Talaromyces marneffei*. *Chiang Mai Med. J.* 2016, 55, 23–30.

52. Pongpom, M.; Vanittanakom, P.; Nimmancee, P.; Cooper, C.R., Jr.; Vanittanakom, N. Adaptation to macrophage killing by *Talaromyces marneffei*. *Future Sci. OA* 2017, 3, Fso215. [CrossRef]
53. Choi, J.H.; Lou, W.; Vancura, A. A novel membrane-bound glutathione S-transferase functions in the intracellular phase of the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 1998, 273, 29915–29922. [CrossRef]
54. Collinson, E.J.; Grant, C.M. Role of yeast glutaredoxins as glutathione S-transferases. *J. Biol. Chem.* 2003, 278, 22492–22497. [CrossRef] [PubMed]
55. Veal, E.A.; Toone, W.M.; Jones, N.; Morgan, B.A. Distinct roles for glutathione S-transferases in the oxidative stress response in *Schizosaccharomyces pombe*. *J. Biol. Chem.* 2002, 277, 35523–35531. [CrossRef] [PubMed]
56. Lee, S.H.; Chung, S.C.; Oh, K.B. GST2 is required for nitrogen starvation-induced filamentous growth in *Candida albicans*. *J. Microbiol. Biotechnol.* 2014, 24, 1207–1215. [CrossRef] [PubMed]
57. Fraser, J.A.; Davis, M.A.; Hynes, M.J. A gene from *Aspergillus nidulans* with similarity to URE2 of *Saccharomyces cerevisiae* encodes a glutathione S-transferase which contributes to heavy metal and xenobiotic resistance. *Appl. Environ. Microbiol.* 2002, 68, 2802–2808. [CrossRef] [PubMed]
58. Burns, C.; Geraghty, R.; Neville, C.; Murphy, A.; Kavanagh, K.; Doyle, S. Identification, cloning, and functional expression of three glutathione transferase genes from *Aspergillus fumigatus*. *Fungal Genet. Biol.* 2005, 42, 319–327. [CrossRef] [PubMed]
59. Weber, S.S.; Parente, A.F.; Borges, C.L.; Parente, J.A.; Bailão, A.M.; de Almeida Soares, C.M. Analysis of the secretomes of *Paecilomyces pullulans* mycelia and yeast cells. *PLoS ONE* 2012, 7, e52470. [CrossRef]
60. Song, J.Y.; Cha, J.; Lee, J.; Roe, J.H. Glutathione reductase and a mitochondrial thioredoxin play overlapping roles in maintaining iron-sulfur enzymes in fission yeast. *Eukaryot. Cell* 2006, 5, 1857–1865. [CrossRef]
61. Bakti, F.; Király, A.; Orosz, E.; Miskei, M.; Emri, T.; Leiter, E.; Pócși, I. Study on the glutathione metabolism of the filamentous fungus *Aspergillus nidulans*. *Acta Microbiol. Immunol. Hung.* 2017, 64, 255–272. [CrossRef]
62. Suizu, T.; Tsutsumi, H.; Ohtake, Y.; Kawado, A.; Imayasu, S.; Kimura, A.; Murata, K. Absolute glutathione requirement for sporulation of a yeast strain. In *Biochemical and Biophysical Research Communications*; Elsevier: Cambridge, MA, USA, 2017, 425, 1151–1154. [CrossRef]
63. Lee, J.C.; Straffon, M.J.; Jang, T.Y.; Higgins, V.J.; Grant, C.M.; Dawes, I.W. The essential and ancillary role of glutathione in *Saccharomyces cerevisiae* analysed using a grande gsh1 disruptant strain. *FEMS Yeast Res.* 2001, 1, 57–65. [CrossRef]
64. Ku, J.W.K.; Gan, Y.H. New roles for glutathione: Modulators of bacterial virulence and pathogenesis. *Redox Biol.* 2021, 44, 102012. [CrossRef] [PubMed]
65. Reniere, M.L.; Whiteley, A.T.; Hamilton, K.L.; John, S.M.; Lauer, P.G.; Portnoy, D.A. Glutathione activates virulence gene expression of an intracellular pathogen. *Nature* 2015, 517, 170–173. [CrossRef]
66. Zhang, Y.; Zhang, C.; Du, X.; Zhou, Y.; Kong, W.; Lau, G.W.; Chen, G.; Kohli, G.S.; Yang, L.; Wang, T.; et al. Glutathione activates type III secretion system through Vir in *Pseudomonas aeruginosa*. *Front. Cell Infect. Microbiol.* 2019, 9, 164. [CrossRef]
67. Pócși, I.; Prade, R.A.; Penninckx, M.J. Glutathione, altruistic metabolite in fungi. *Ado. Microb. Physiol.* 2004, 49, 1–76. [CrossRef] [PubMed]
68. Jürgensen, C.W.; Jacobsen, N.R.; Emri, T.; Eriksen, S.H.; Pócși, I. Glutathione metabolism and dimorphism in *Aureobasidium pullulans*. *J. Basic Microbiol.* 2001, 41, 131–137. [CrossRef]
69. Manavathu, M.; Gunasekaran, S.; Porte, Q.; Manavathu, E.; Gunasekaran, M. Changes in glutathione metabolic enzymes during yeast-to-mycelium conversion of *Candida albicans*. *Can. J. Microbiol.* 1996, 42, 76–79. [CrossRef]
70. Thomas, D.; Klein, K.; Manavathu, E.; Dimmock, J.R.; Mutus, B. Glutathione levels during thermal induction of the yeast-to-mycelial transition in *Candida albicans*. *FEMS Microbiol. Lett.* 1991, 61, 331–334. [CrossRef]
71. Pócși, I.; Molnár, Z.; Puszthahelyi, T.; Vareczka, Z.; Emri, T. Yeast-like cell formation and glutathione metabolism in autolysing cultures of *Penicillium chrysogenum*. *Acta Biol. Hung.* 2007, 58, 431–440. [CrossRef]
72. Guedouari, H.; Gergondrey, R.; Bourdais, A.; Vanparis, O.; Bulteau, A.L.; Camadrow, J.M.; Aucière, F. Changes in glutathione-dependent redox status and mitochondrial energetic strategies are part of the adaptive response during the filamentation process in *Candida albicans*. *Biochim. Biophys. Acta* 2014, 1842, 1855–1869. [CrossRef]
73. Maras, B.; Angioliela, L.; Mignogna, G.; Vavala, E.; Macone, A.; Colone, M.; Pitari, G.; Stringaro, A.; Dupré, S.; Palamara, A.T. Glutathione metabolism in *Candida albicans* resistant strains to fluconazole and micafungin. *PLoS ONE* 2014, 9, e98387. [CrossRef] [PubMed]
74. González-Párraga, P.; Marín, F.R.; Argüelles, J.C.; Hernández, J.A. Correlation between the intracellular content of glutathione and the formation of germ-tubes induced by human serum in *Candida albicans*. *Biochim. Biophys. Acta* 2005, 1722, 324–330. [CrossRef] [PubMed]
75. Carlin, A.F.; Beyhan, S.; Peña, J.F.; Stajich, J.E.; Vireyakosol, S.; Fierer, J.; Kirkland, T.N. Transcriptional analysis of *Coccidioides immitis* mycelia and spherules by RNA sequencing. *J. Fungi* 2021, 7, 366. [CrossRef] [PubMed]
76. Kawado, A.; Suzu, T.; Imayasu, S.; Shigematsu, T.; Kimura, A.; Murata, K. Highly efficient sporulation induced by glutathione or glutathione thiol esters in sake (Kyokai no. 7) and a wild-type yeast. *J. Ferment. Bioeng.* 1992, 74, 363–367. [CrossRef]